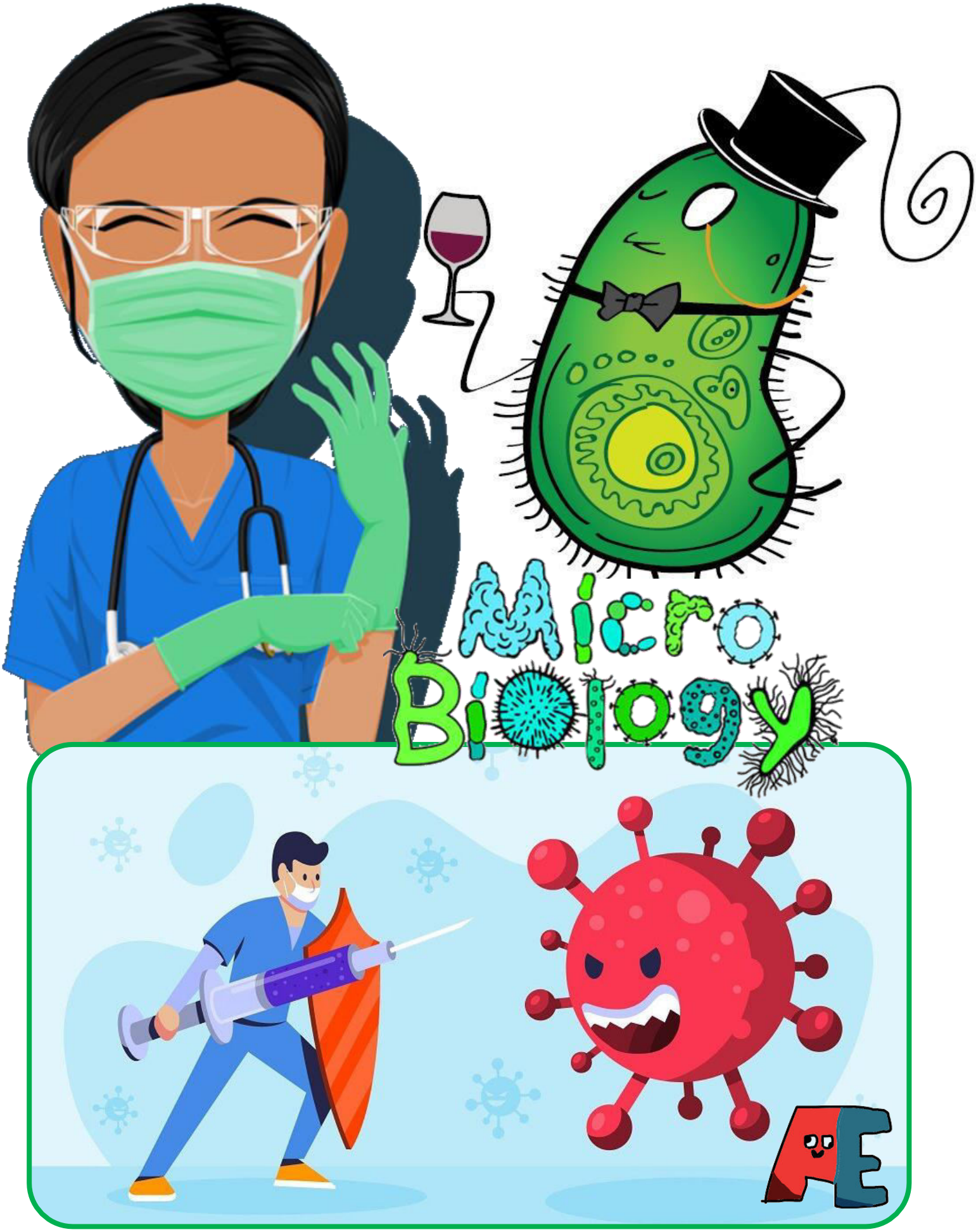

Micro
Biology

General MICROBIOLOGY

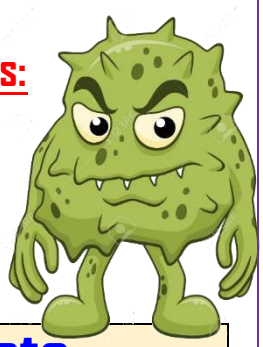
PART

Chapter 1

Introduction To Microorganisms

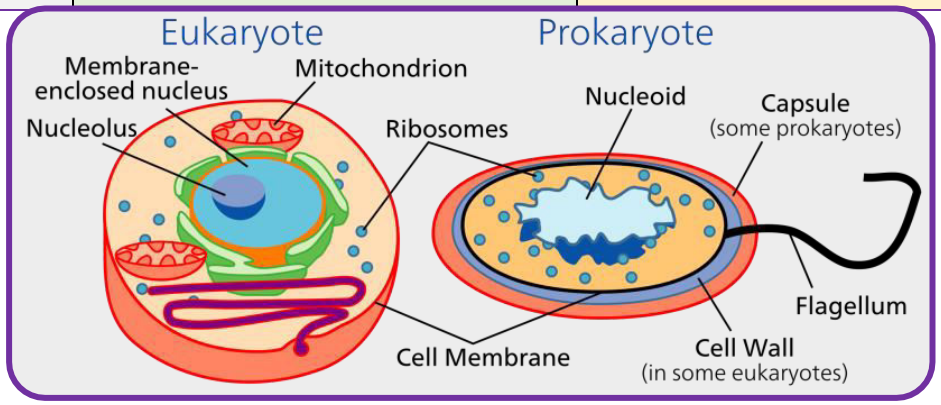
- **Microorganisms are generally unicellular** → the whole organism is one cell.
- a single microbial cell performs all the functions required to maintain itself and propagate.

Microorganisms may be classified in the following large biological groups:



1. Algae.
2. Protozoa.
3. Slime molds.
4. Fungi.
5. Bacteria.
6. Archaebacteria.
7. Viruses.
8. Prions.

	Prokaryote	Eukaryote
	Pro = before	**EU** = True
Size	Smaller	Larger
Development	Simple	More developed
Nucleus	**No True nucleus** One chromosome is called ***Nucleoid*** or Nuclear region	**True nucleus**
Nuclear membrane	No nuclear membrane	Surrounded by nuclear membrane
Mitochondria	No mitochondria	Contain mitochondria
Organelles	No membrane bound organelles	Contain membrane bound organelles
Ribosomes	**70S**	**80S**
Cytoplasmic membrane	No sterol (**except mycoplasma**)	Contain sterol
Examples	Bacteria Mycoplasma Rickettsia Chlamydia **Blue green** algae Archaebacteria	Algae (**except blue green**) Protozoa Slime molds Fungi Plants Animals



Viruses	☠ Smallest infective agent ☠ Have no cell structure ☠ **Obligate intracellular parasites** → require host cells
Viroids	Single stranded RNA (**no protein**) Causes diseases in plants
Prions	Infectious protein particles (**no nucleic acids**)
Blue green algae	Do not cause infections May produce potent toxins (drinking polluted waters)

MCQs

1- Which of the following microorganisms has a nuclear membrane?

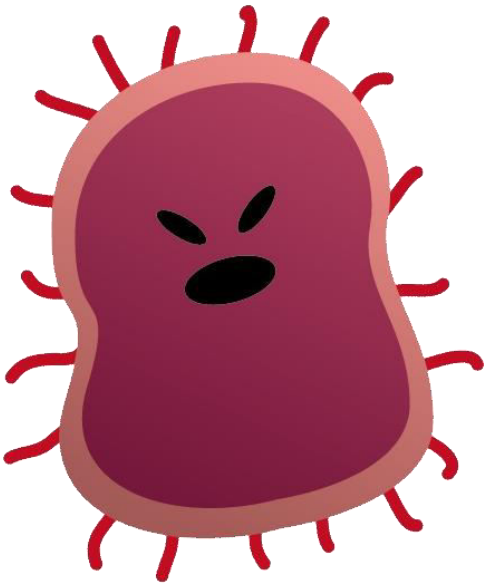
a- Viruses
b- Fungi
c- Prions
d- Bacteria
e- Viroids

2- Viruses have all the following characteristics EXCEPT:

a- They are one of the smallest infectious agents.
b- They have no cell structure.
c- They are obligate intracellular parasites.
d- They require the host biological machinery for their replication.
e- They are prokaryotic.

3- Prions:

a- Are single stranded circular RNA
b- Are devoid of proteins
c- Are infectious proteins devoid of nucleic acids
d- Are prokaryotic cells
e- Cause diseases in plants



Chapter 2

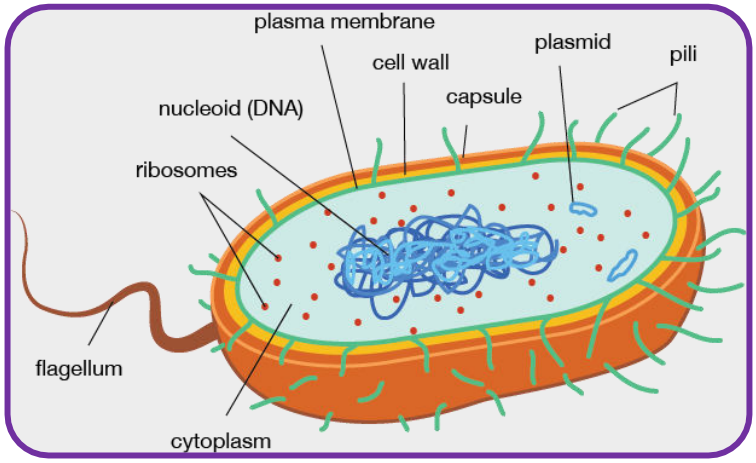
BACTERIA: THEIR STRUCTURE AND ORGANIZATION

- Bacteria were first discovered by ***Leeuwenhoek*** ***1674***.
- They are among the most widely distributed forms of life.
- They are found in air, water and soil.
- They are also found in or on the human body, animals and plants.

Bacterial Morphology

Bacteria are classified based on their morphological features such as:

1) Shape
2) Size
3) arrangement
4) staining characteristics.

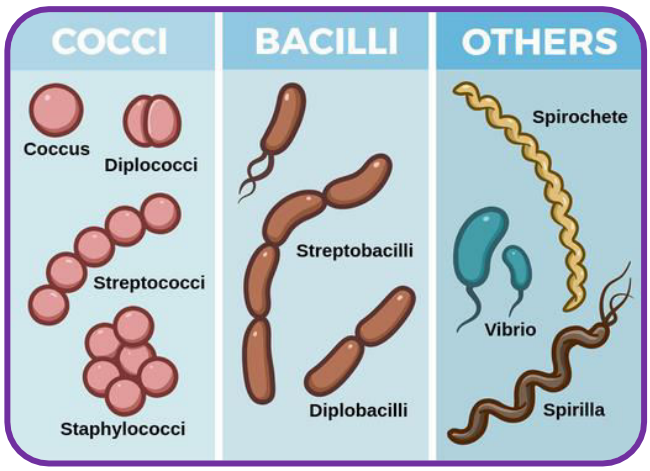


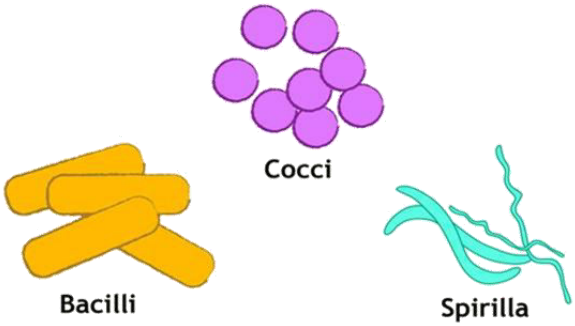
Bacterial Size

Most bacteria range in size from **0.2-1.2 µm** in width and **0.4-14 µm** in length.

Bacterial Shape and Arrangement:

	Cocci	Bacilli	Spiral bacteria
Definition	Spherical organisms **Singular** = coccus	Rod shaped (**Stick**) **Singular** = bacillus	
Types	**Diplococci**: pairs of cells → *Neisseria*. **Irregular grape-like clusters**, → *staphylococci*. **Chains of four or more** → *streptococci*.	May occur single, in pairs, or chains **Coccobacilli**: Short **Vibrio**: curved	**Spirilla**: rigid **Spirochetes**: flexible

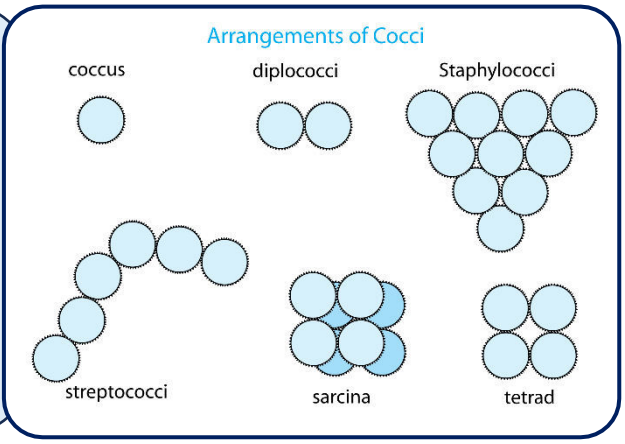




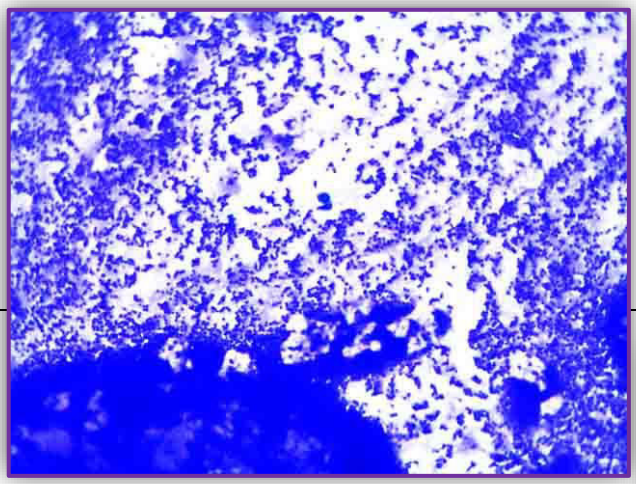
The arrangement of cells is determined by the planes of division.

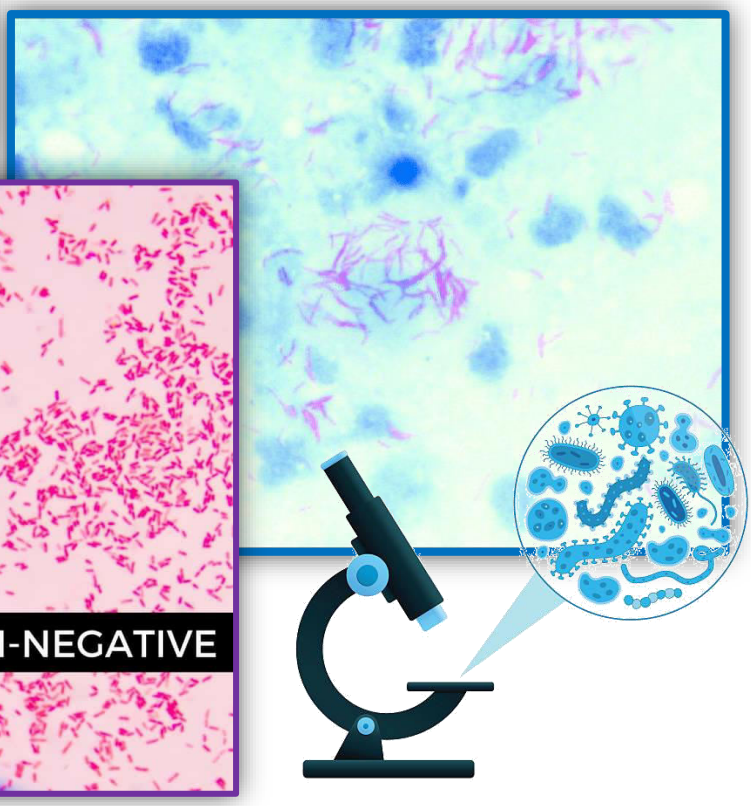
the cocci that divide along a single plane produce diplococci or chains → streptococci

those that divide on many planes produce clusters → staphylococci.



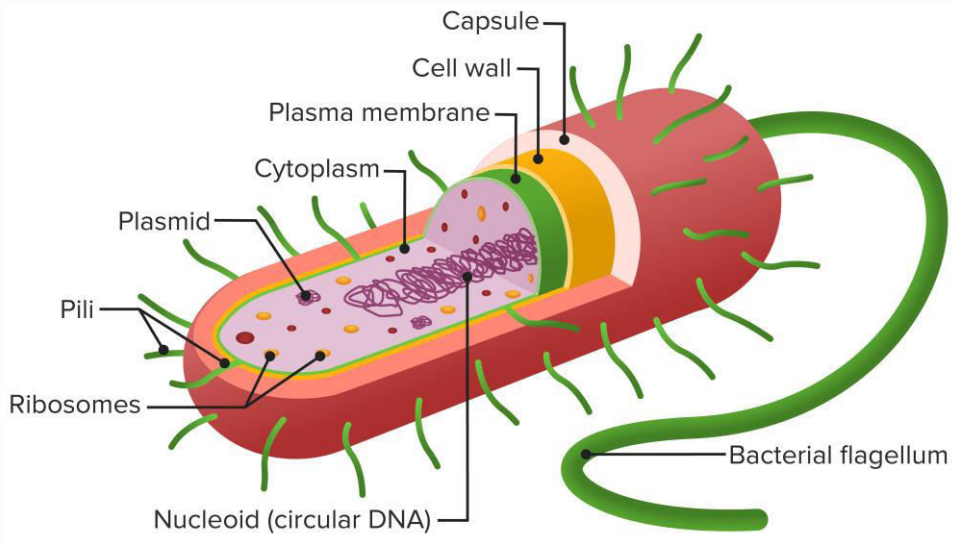
Staining Characteristics

Simple staining	Differential staining
Using a single dye	Requires more than one dye
Stained structures give the same color	**Distinguish between** different types of bacteria by giving different colors
Used for revealing the characteristics of ***size***, ***shape***, ***arrangement*** 	**Gram stain:** The most important stain in microbiology **Divides bacteria into:** 1- gram positive (violet) 2- gram negative (red) **Ziehl-neelsen stain:** Used to stain **mycobacteria** (acid fast bacilli)



Bacterial Ultra-Structures and their Functions

All bacteria have a nucleoid, ribosomes and a cytoplasmic membrane.
Most bacteria also have a cell wall and some are further enveloped by a capsule or slime layer.
Some types of bacteria have also various appendages as flagella and pili.



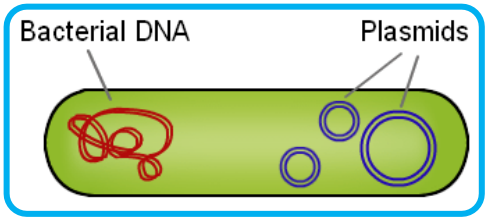
Cytoplasm

Few morphologically distinct components can be found within the cytoplasm

Nucleoid:

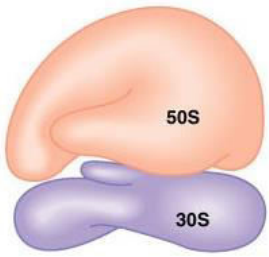
- Genetic information of a bacterial cell is contained in a **single** circular molecule of double-stranded DNA, which constitutes the bacterial chromosome.
- It is 1 mm long and is packed into a supercoiled state inside the cell.

Plasmids:



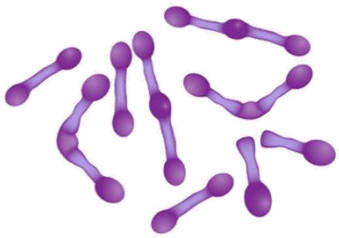
- In many bacteria, **additional genetic information** is contained on plasmids which are small circular extrachromosomal DNA molecules that can replicate independently of the chromosome.

Ribosomes:



- They are the **site of protein synthesis** in the cell.
- Ribosomes consist of protein and RNA.
- Prokaryotic ribosomes have a sedimentation constant of **70S**, smaller than the **80S** ribosomes of eukaryotes.
- This difference makes bacterial ribosomes a selective target for antibiotic action.

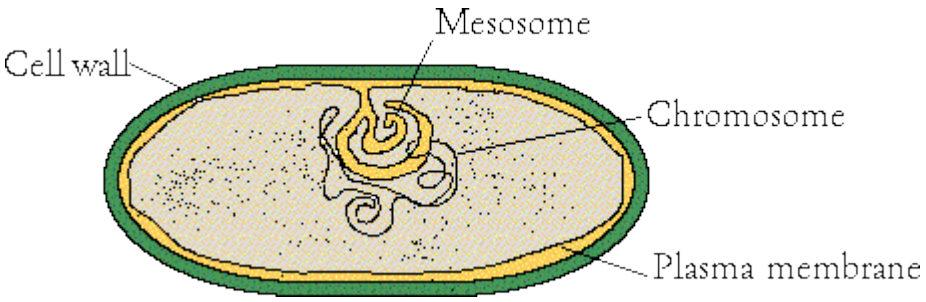
Inclusion granules:



- These are **granules of nutrient materials**, usually phosphates, sulphur, carbohydrates and lipids.
- Energy reserves are usually stored as glycogen, starch or poly-β-hydroxybutyrate.
- Phosphate is stored in **metachromatic** or **volutin granules**, which are used for synthesis of ATP.

Mesosomes:

- These are complex **invagination of the cytoplasmic membrane**.
- They are involved in cell division and sporulation.
- They also have a function analogous to the mitochondria in eukaryotes providing a membranous support for respiratory enzymes



Cytoplasmic Membrane

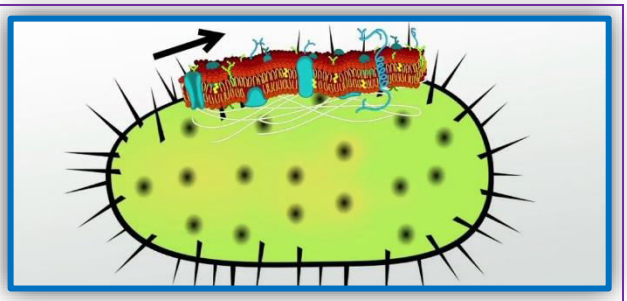
Definition: It is a phospholipid protein bilayer similar to that of eukaryotic cells except that, in bacteria, it lacks sterols.

It has the following functions:

1. **Selective transport**:
molecules move across the cytoplasmic membrane by simple diffusion, facilitated diffusion and active transport.

2. **Excretion of extracellular enzymes:**
   a. **Hydrolytic enzymes**: which digest large food molecules into subunits small enough to penetrate the cytoplasmic membrane.
   b. **Enzymes used to destroy harmful chemicals**
      Example: penicillin-degrading enzymes.

3. **Respiration**: The respiratory enzymes are located in the cytoplasmic membrane, which is thus a functional analogue of the mitochondria in eukaryotes.



4. **Cell wall biosynthesis**:

The cytoplasmic membrane is the site of:

a. The enzymes of cell wall biosynthesis.
b. The carrier lipids on which the subunits of the cell wall are assembled.

5. **Reproduction**:

- A specific protein in the membrane attaches to the DNA and separates the duplicated chromosomes from each other.
- A septum forms by the cytoplasmic membrane to separate the cytoplasm of the two daughter cells.

6. **Chemotactic system:**

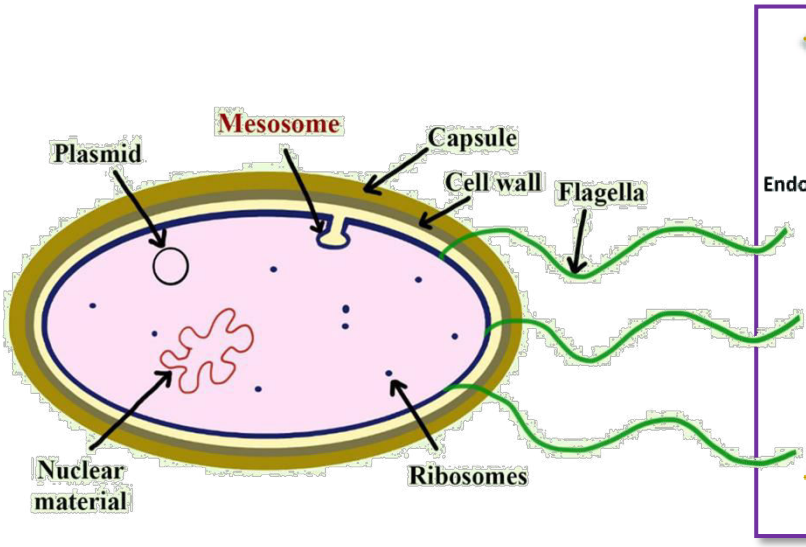
- Attractants and repellants bind to specific receptors in the cytoplasmic membrane and send signals to the cell's interior.
- The cell then responds to the surface message.

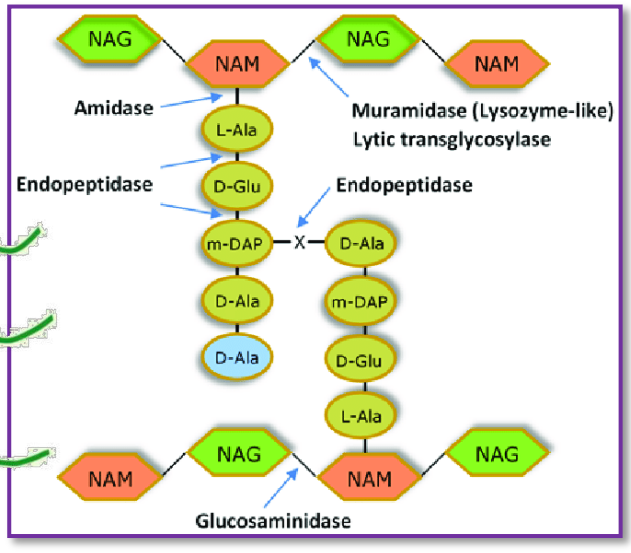
Cell Wall

- The bacterial cell wall is the structure that immediately surrounds the cytoplasmic membrane.
- It is **10-25 nm** thick strong and relatively **rigid**, though having some elasticity

Structure of the cell wall

- The cell wall of bacteria is a complex structure.
- Its impressive strength is primarily due to **peptidoglycan** (murein or mucopeptide).
- **Peptidoglycan**: a complex polymer consisting of N-acetylglucosamine (**NAG**) and N-acetylmuramic acid (**NAM**) unique to bacteria.
- A set of identical **tetrapeptide side chains** are attached to NAM.

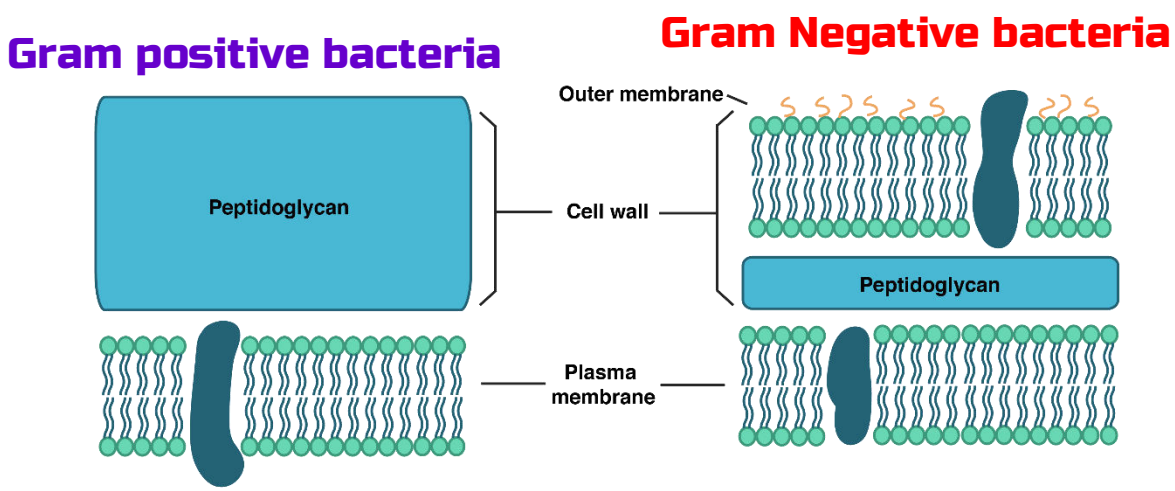




Gram-positive cell wall:

1. **Peptidoglycan**:
   - There are as many as **40 sheets** of peptidoglycan.
   - comprising up to **50%** of the cell wall material.
   - Despite the thickness of peptidoglycan, chemicals can readily pass through.

2. **Teichoic acids:**
   - They are the polymer of ribitol or glycerol phosphate.
   - They are found in the cell wall of most Gram-positive bacteria.
   - Teichoic acids and cell wall associated proteins are the major **surface antigens** of the Gram-positive bacteria.



Gram-negative cell wall:

1. **Peptidoglycan**:
   - It is much **thinner**.
   - composed of **only one or two sheets**.
   - comprising **5-10%** of the cell wall material.

2. **Outer membrane**:
   - It is phospholipid protein bilayer present external to the peptidoglycan layer.
   - The outer surface of the lipid bilayer is composed of molecules of lipopolysaccharide (**LPS**) which consists of a complex lipid called **lipid A** chemically linked to polysaccharides.
   - **Lipid A** of the LPS forms the **endotoxin** of the Gram-negative bacteria, while polysaccharides are the outermost molecules of the cell wall and are **major surface antigens** of the Gram-negative bacterial cell (somatic or O antigen).

3. **Periplasmic space**:
   - It is the space between the cytoplasmic and outer membranes.
   - It contains the peptidoglycan layer and a gel-like solution of proteins.

Functions of the cell wall

1) It maintains the characteristic **shape** of the bacterium.
2) It **supports** the weak cytoplasmic membrane against the high internal osmotic pressure of the protoplasm (**5-25 atm**.).
3) It plays an important role in **cell division**.
4) It is responsible for the **staining affinity** of the organism.

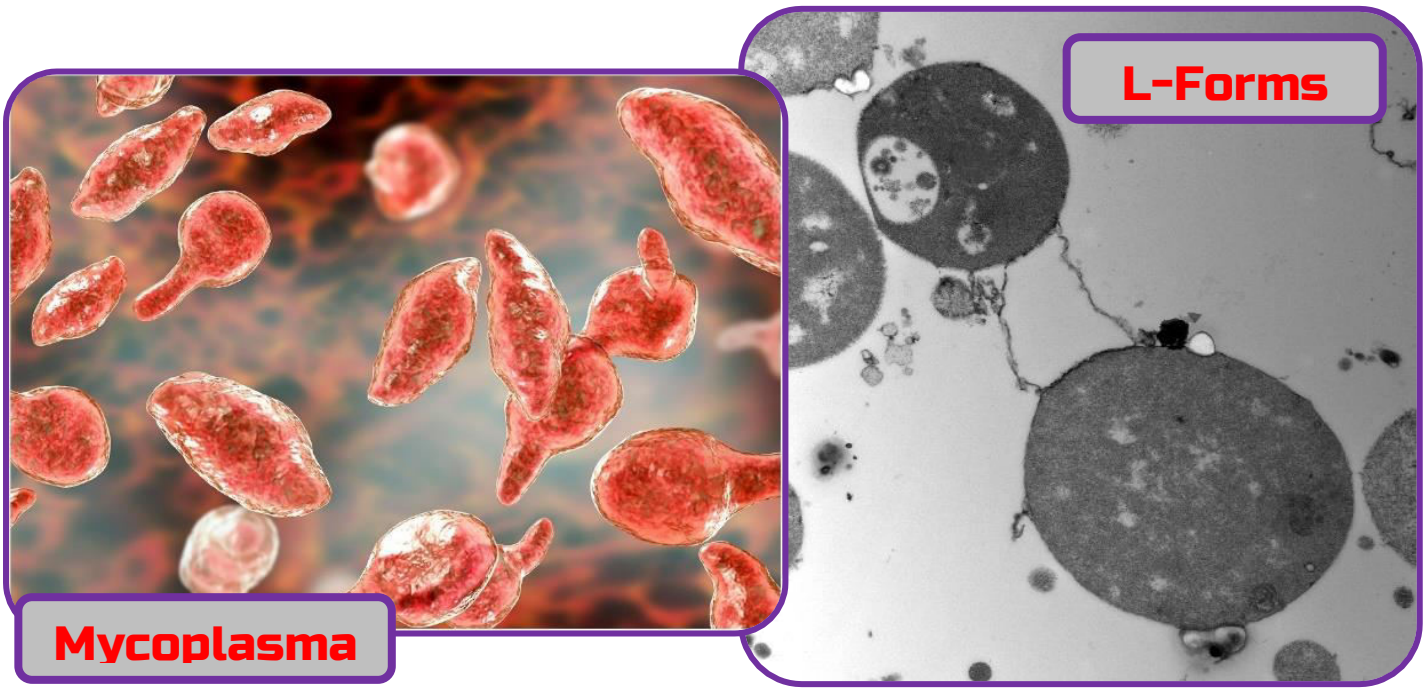
Cell Wall deficient Bacteria

Mycoplasma:

- It is the only group of bacteria that exists naturally **without cell wall**.
- Mycoplasmas do not assume a defined recognizable shape, because they lack a rigid cell wall.
- These organisms are **naturally resistant** to cell wall inhibitors, such as penicillin and cephalosporins.

L. Forms:

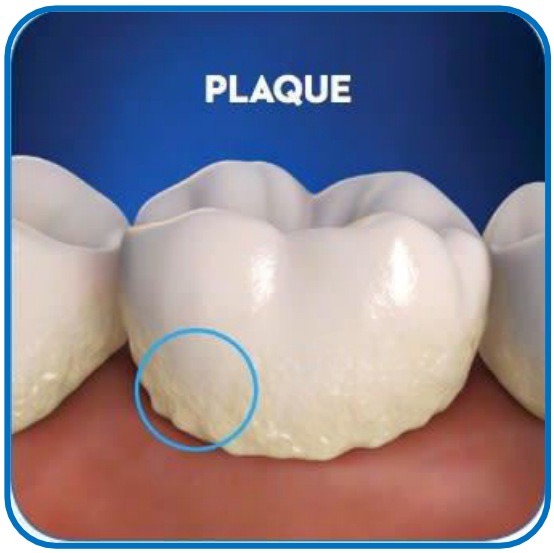
- They are wall defective or wall deficient bacteria.
- **"L"** stands for Lister Institute in London, where they were first discovered.
- **L- Forms** may develop from cells that normally possess cell wall, when they are exposed to hydrolysis by lysozyme or by blocking peptidoglycan biosynthesis with antibiotics, such as penicillin, provided that they are present in an isotonic medium.
- **Some L. forms resynthesize their walls** once the inducing stimulus is removed. **Others** → permanently lose the capacity to produce a cell wall.
- L. forms may survive antibiotic therapy.
- Their reversion to the walled state can produce relapses of the overt infection.

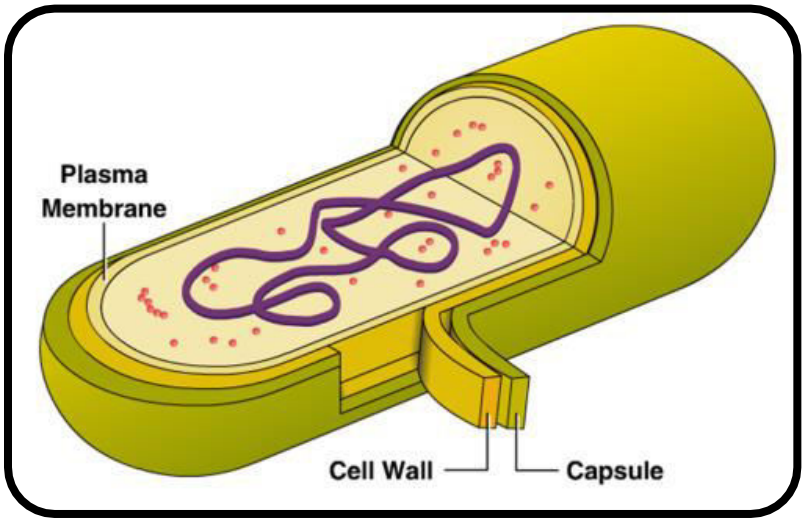


Capsule and Related Structures

- Many bacteria synthesize large amount of extracellular polymer that collects outside the cell wall to form an additional surface layer.
- This layer is formed **only inside the host** *(in-vivo)*.
- **All capsules are Made of polysaccharides except Bacillus anthracis** (polypeptide).

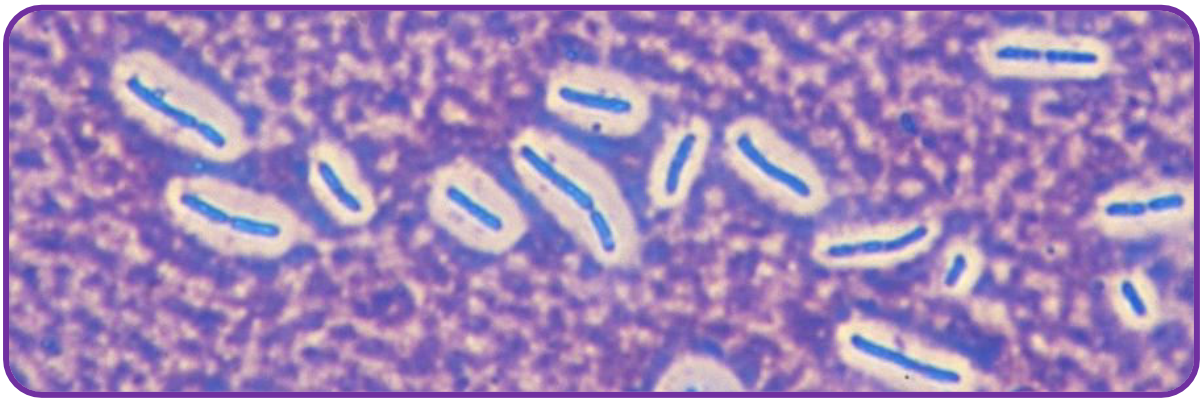
1) **Capsule**: It is such a layer that adheres to the surface of the cell and forms a well-defined halo when differentially stained, to be resolved with the light microscope.
2) **Slime layer**: It is a surface layer that is loosely distributed around the cell.
3) **Glycocalyx**: It is a loose meshwork of polysaccharide fibrils extending outwards from the cell.





Functions:

1. **It protects the cell wall against various kinds of antibacterial agents**
   Examples: bacteriophages, colicins, complement and lysozymes.
2. **It protects the bacterial cell from phagocytosis**.
   the capsule is considered an important virulence factor.
3. **Some bacteria attach to the target surface by using their capsules or glycocalyx in order to establish infection.**
   Streptococcus mutans form glycocalyx with which the bacteria stick to the tooth enamel.



Appendages

- Several Structures project through the cell wall of bacteria to form surface appendages.
- **The most commonly observed** are **flagella** and **Pilli**.

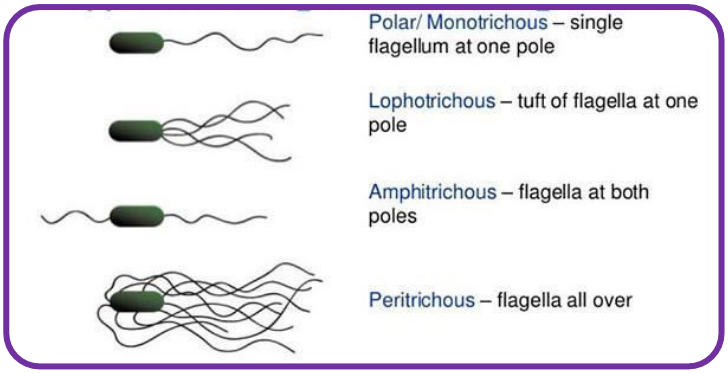
Flagella

Many genera of bacteria move by means of flagella.

- Flagella are only **20 nm** in diameter.
  too small to be detected by Light microscope.
  They can be demonstrated clearly with the electron microscope.

- **The location and number of flagella on a cell vary according to bacterial species.**

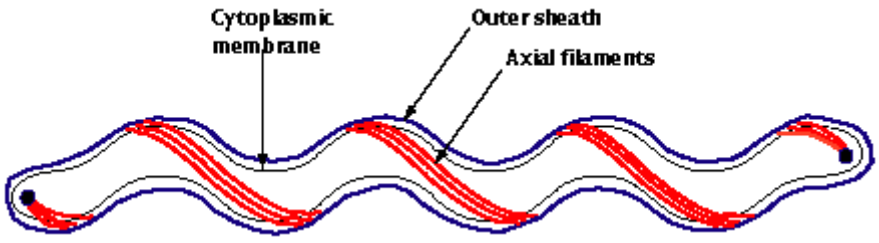
1) **Monotrichous** (single polar flagellum)
2) **lophotrichous** (multiple polar flagella)
3) **peritrichous** (flagella distributed over the entire cell)



- Flagella consist of a single type of protein called **flagellin** which differs in different bacterial species.
- **The flagellins are highly antigenic** (**H antigen**).
- Motile bacteria tend to migrate towards regions where there Is a higher concentration of nutrients and solutes (**chemotaxis**) and away from disinfecting substances (**negative chemotaxis**).

Axial filaments

- **composed of** two groups of fibers that originate within the opposite ends of the cell and overlap in the middle.
- **Structurally and chemically**, the fibers of the axial filaments are similar to flagella and they are sometimes called "**endoflagella**".
- **Spirochaetes** move by means of these axial filaments.
- When the cell moves → It rotates around Its longitudinal axis and flexes and bends along Its length.



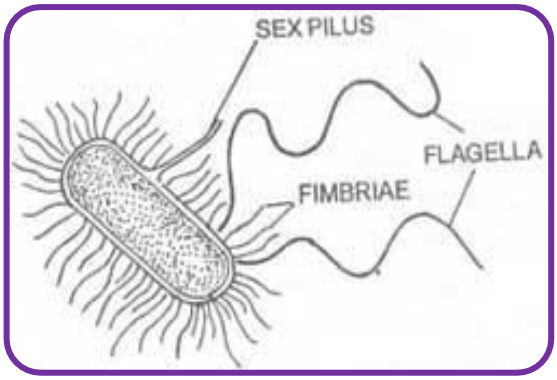
Pilli (Fimbriae)

singular: pilus

- **Definition**: are protein tubes that extend from the cells.
- They are shorter and thinner than flagella.
- can be observed only by the electron microscope.
- They are composed of structural protein subunits termed **pillins**.

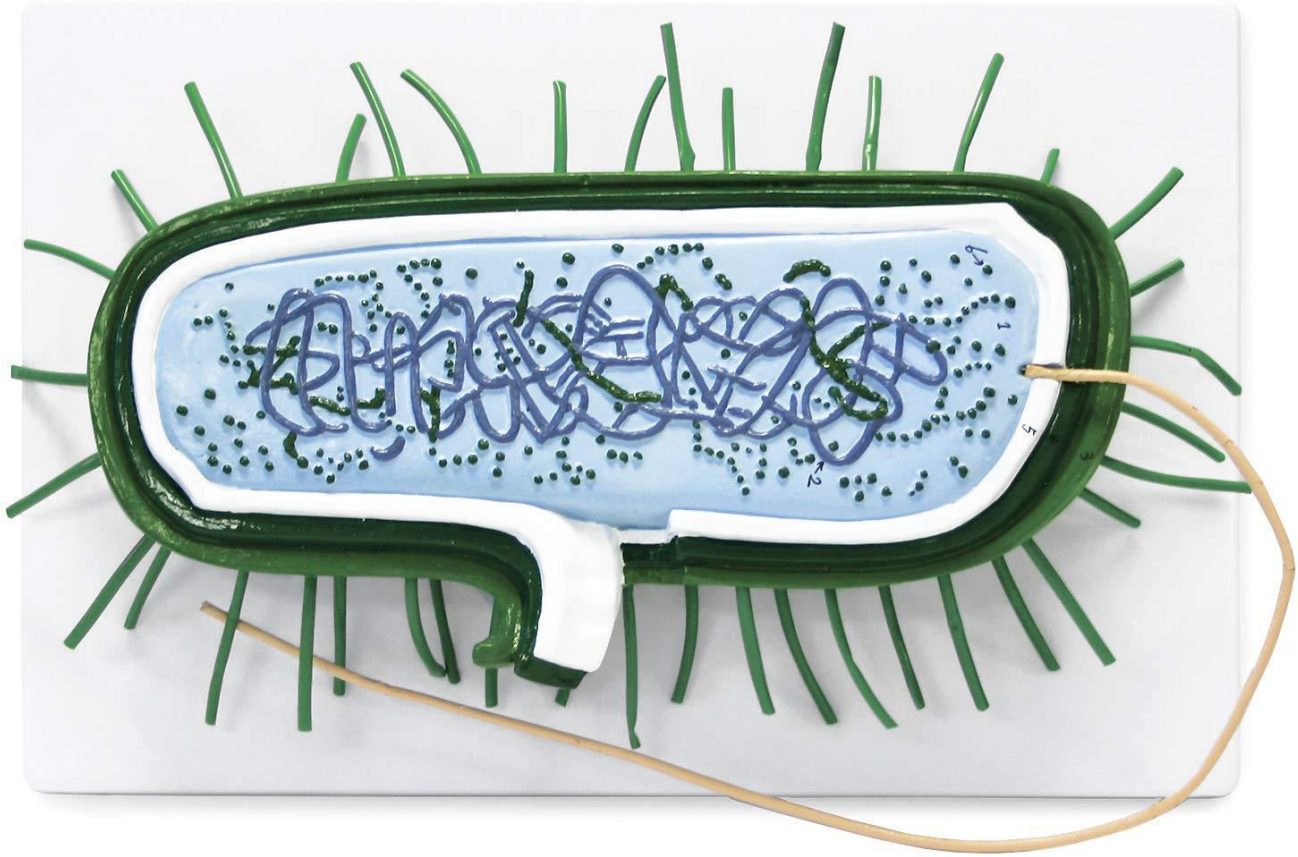
Functions:

1. **Adherence**: It is the function of the short pill (**fimbria**) that occur in great numbers around the cell.
   They enable bacteria to attach to the surfaces, contributing to the establishment of Infection → **virulence factor**.

   Examples → ***N. gonorrhoeae*** withstands the flushing action of urine by adhering to the urethral mucosa.

2. **Conjugation**: A special long pilus called the **sex pilus** (**F pilus**) Is Involved In the transfer of DNA between bacteria, a process known as conjugation



Bacterial Spores (Endospores)

- Some bacteria (***Bacillus*** and ***Clostridium***) develop a highly resistant resting phase or endospore that does not grow or reproduce, and exhibits absolute **dormancy**.
- **A single vegetative bacterium** forms a single spore by a process called sporulation.
- **A single vegetative bacterium** emerges from a spore during germination.

Sporulation

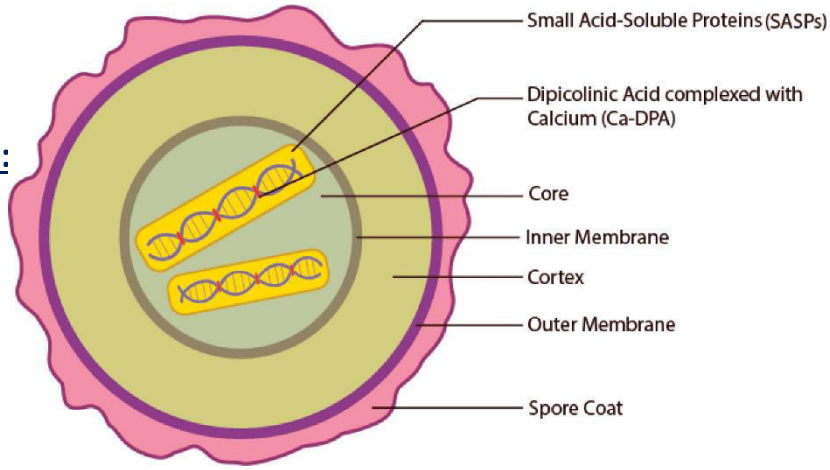
- **Sporulation is triggered by the onset of unfavorable environmental conditions:**
  1) depletion of nutrients.
  2) accumulation of metabolites.
  3) changes in the growth requirements (moisture, temperature, pH, or oxygen tension).

- **The cytoplasmic membrane invaginates enclosing a section of the cytoplasm that contains**:
  1) The bacterial chromosome.
  2) Some ribosomes.
  3) other cytoplasmic materials that will be needed for germination.
- **It acquires** a thick cortex and a thin but tough outer spore coat.

Viability and resistance

Spores are much more resistant to:

1) Disinfectants
2) drying
3) heating.



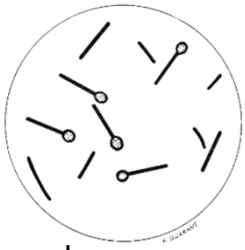
Moist heat at 121°C for 10-20 minutes is needed to kill spores while 60°C suffices to kill vegetative forms.

The marked resistance of the spores has been attributed to several factors:

1. Thermal resistance is provided by their high content of $Ca^{2}+$ and **dipicolinic acid** (a compound unique to endospores).
2. The impermeability of their cortex and outer coat.
3. Their low content of water.
4. Their very low metabolic and enzymatic activity.

Germination

- ☹ **Endospores respond quickly** to environmental changes returning to the vegetative state within 15 min.
- ☹ In the process of germination, the spores absorb water and swell, the protective coat disintegrates and a single vegetative cell emerges.

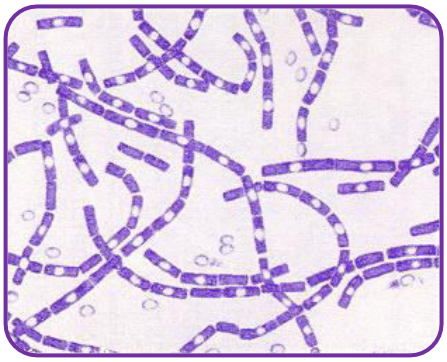


Morphology

1- **Staining**:
   - ❖ Using Gram's stain
   - ❖ the spore **remains uncolored** and can be seen as a clear area within the stained cell.
   - ❖ The spores can be stained using special procedures.

2- **The position**:
   - ❖ In relation to the body of the bacillus, the spore may be central, terminal or subterminal.

3- **The shape**:
   - ❖ The spores may be **oval** or **rounded**.



The position and shape of spores are characteristic of the species and may help in the microscopic identification of the bacterium

MCQs

1- The following are functions of the cytoplasmic membrane EXCEPT:

a- Respiration
b- Cell wall biosynthesis
c- Reproduction
d- Staining affinity
e- Selective transport

2- Lipid A is a cell wall component of:

a- Gram positive bacteria
b- Gram negative bacteria
c- Fungi
d- Algae
e- Viruses

3- One of the following is a function of the cell wall:

a- Maintaining the characteristic shape of the bacterial cell
b- Selective transport
c- Respiration, since respiratory enzymes are located in it
d- Protein synthesis
e- Excretion of extracellular enzymes

4- All the following are characters of L-forms of bacteria EXCEPT:

a- They are naturally occurring bacteria without cell wall.
b- They are resistant to antibiotics which inhibit cell wall synthesis.
c- They develop only in isotonic media.
d- They can produce relapses of overt infections.
e- They may resynthesize the cell wall.

5- Bacteria are protected from phagocytosis by:

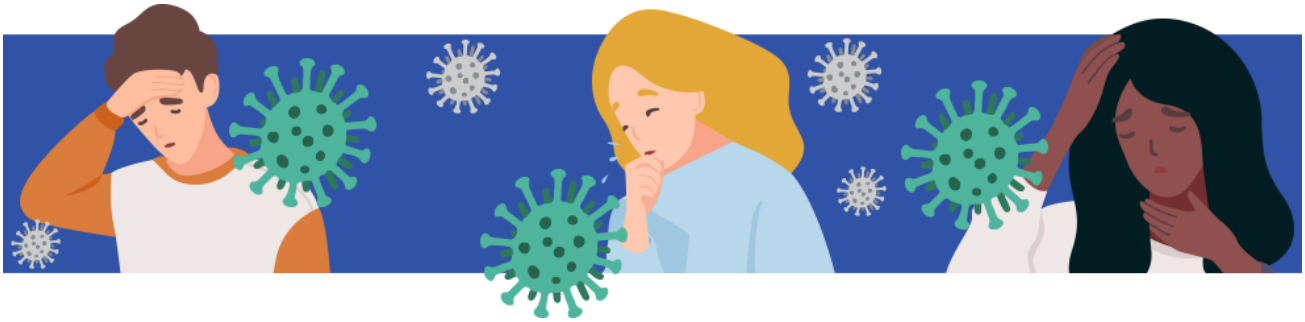
a- The capsule
b- Lipoprotein
c- The mesosome
d- The outer membrane
e- Peptidoglycan

6- All of the following are true concerning pili EXCEPT:

a- They mediate bacterial adherence.
b- They may be involved in bacterial conjugation.
c- Their antigen is called H antigen.
d- They are important virulence factors.
e- They are protein in nature.

7- The marked resistance of the spores can be attributed to all the following factors EXCEPT:

a- The impermeability of their cortex and outer coat
b- Their ability to resist phagocytosis
c- Their low content of water
c- Their very low metabolic and enzymatic activity
d- Their high content of Ca and dipicolinic acid



Chapter 3

Bacterial Growth and Physiology

Growth involves an increase in the size and number of organisms.

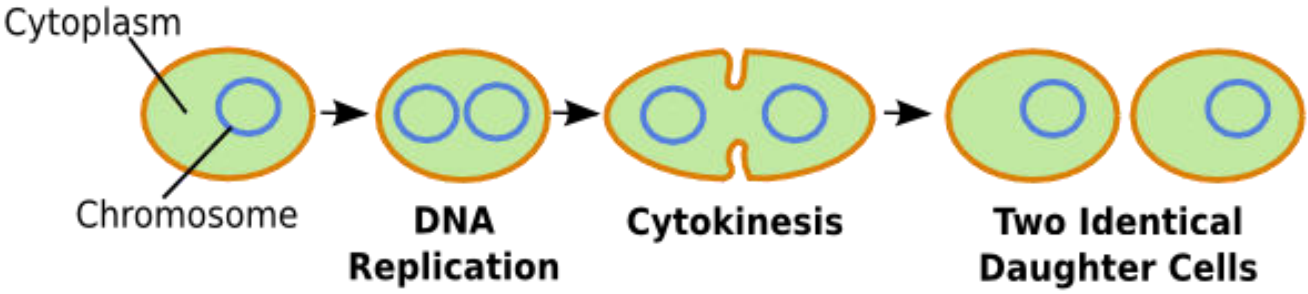
In the laboratory, bacterial growth can be seen in one of two main forms:

1. **Development of colonies**, which are the macroscopic products of **20-30** cell divisions of a single bacterium on solid media.
2. **Transformation of a clear fluid medium to a turbid suspension**.

Bacterial Reproduction

Bacterial multiplication takes place by simple binary fission:

1) The cell grows in size, usually elongates.
2) The bacterial chromosome acts as a template for the replication of another copy.
3) Each copy becomes attached to a mesosome on the cytoplasmic membrane.
4) The protoplasm becomes divided into two equal parts by the growth of a transverse septum from the cytoplasmic membrane and cell wall.

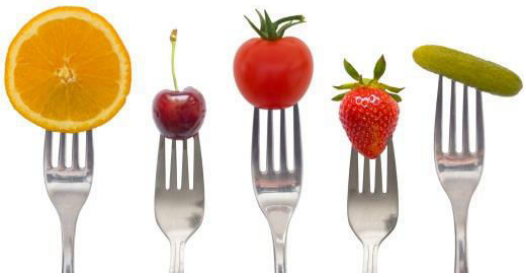


In some species, this septum splits the parent cell completely into two separate daughter cells.
In others, the cell walls of the daughter cells remain continuous for some time after division giving the characteristic arrangement → pairs, clusters or chains.

Generation time (doubling time):

- **is the time between two successive divisions.**
- It may be as short as **13 min** in ***Vibrio cholerae***
  may reach **24 h**. in ***Mycobacterium tuberculosis***.

Growth Requirements



bacteria need the following growth requirements:

1-Nutrients:

According to the means by which a particular organism obtains energy and raw material to sustain its growth, **bacteria are classified into**:

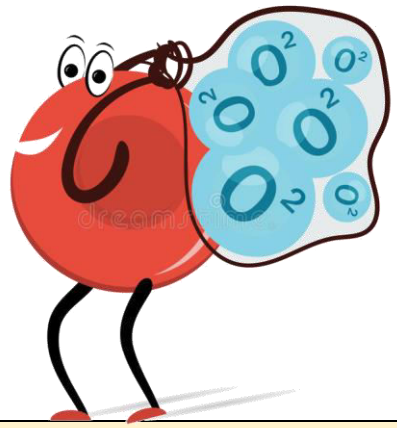
Autotrophs	Heterotrophs
They can utilize simple inorganic materials (CO_2) as a source of carbon and **ammonium salts** as a source of nitrogen.	require organic source of carbon
They can synthesize complex organic substances from the simple inorganic materials.	cannot synthesize organic complex substances
The energy required for their metabolism is predominantly derived from light or simple chemical reactions.	
Autotrophs are of no or little medical importance	**most bacteria of medical importance** are heterotrophs

2-Oxygen (O_2) :

According to O_2 requirements, bacteria are classified into:

Type	Definition	Examples
Strict (obligate) aerobes	**Require oxygen** for growth	**Pseudomonas aeruginosa**
Strict (obligate) anaerobes	**Require complete absence of oxygen**	**Bacteroides fragilis**
Facultative anaerobes	**grow better in presence of oxygen** but still are able to grow in its absence	**Staphylococci, E. coli (Most medically important bacteria)**
Micro-aerophilic organisms	**Require reduced oxygen level**	**Campylobacter and Helicobacter**
Aerotolerant anaerobes	have an anaerobic pattern of metabolism but **can tolerate the presence of oxygen** because they possess superoxide dismutase	**Clostridium perfringens**

Respiration and energy production:



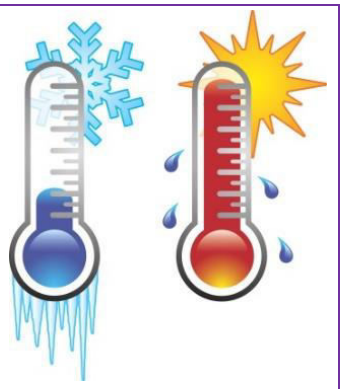
- The cellular respiration is another name of glucose catabolism.
- When it takes place in presence of oxygen, it is called **aerobic cellular respiration**.
- When it takes place in absence of oxygen, it is called **anaerobic cellular respiration**.

aerobic cellular respiration	anaerobic cellular respiration
The glucose catabolism under aerobic conditions	occurs in the absence of oxygen
The final electron acceptor is molecular O_2	The final electron acceptor is an inorganic molecule such as → nitrate (NO_3-), sulfate (SO_4^{-2}), or CO_2.
Results in the production of energy in the form of **38 ATP** molecules	The net yield of ATP molecules is less than it is with aerobic cellular respiration because nitrate, sulfate, and CO_2 are not as good at accepting electrons as oxygen.
During this type of respiration, superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are formed. **These molecules are highly toxic**.	
To cope with this, aerobic organisms have developed two enzymes → **superoxide dismutase** and **catalase**, which detoxify these molecules.	**Compared to aerobes** → obligate anaerobes lack superoxide dismutase and catalase and so they cannot grow in presence of O_2.

Fermentation: **It is an anaerobic process**, because it takes place in the absence of oxygen.
It is used by facultative anaerobes when they exist in an environment that does not contain a suitable inorganic final electron acceptor (NO_3-, SO_4^{-2} or CO_2).
This is the least efficient method of generating energy.

3 . Carbon dioxide (CO_2):

- The minute amount of CO_2 present in air is sufficient for most bacteria.
- certain species require higher concentrations (**5-10%**) of CO_2 for growth (**capnophilic**) → ***Neisseria spp.*** and ***Brucella abortus.***



4. Temperature:

A. **Mesophiles**
- are organisms able to grow within a temperature range of **20-40°C**.
- Pathogens which replicate on or in human body are able to grow within this range, with an optimum temperature of 37°C which is the normal body temperature.

B. **Psychrophiles** (**cold-loving**)
- are capable of growth at refrigeration temperature (**0-80C**)
- Example: *Flavobacterium spp.·*

C. **Thermophiles** (**Heat-loving**)
- grow best at high temperature **(>60°C**)
- Example: *Bacillus stearothermophilus.*

5 . Hydrogen ion concentration (pH):

- Most microorganisms of clinical significance grow best in media whose pH is close to that of human body (**pH 7.2**).

- **some microorganisms grow better at:**
  1- **an alkaline pH** (**8-9**) → ***V. cholerae***.
  2- **acidic pH** (**4 or less**) → ***Lactobacilli***.

Growth Phases (Bacterial Growth Curve)

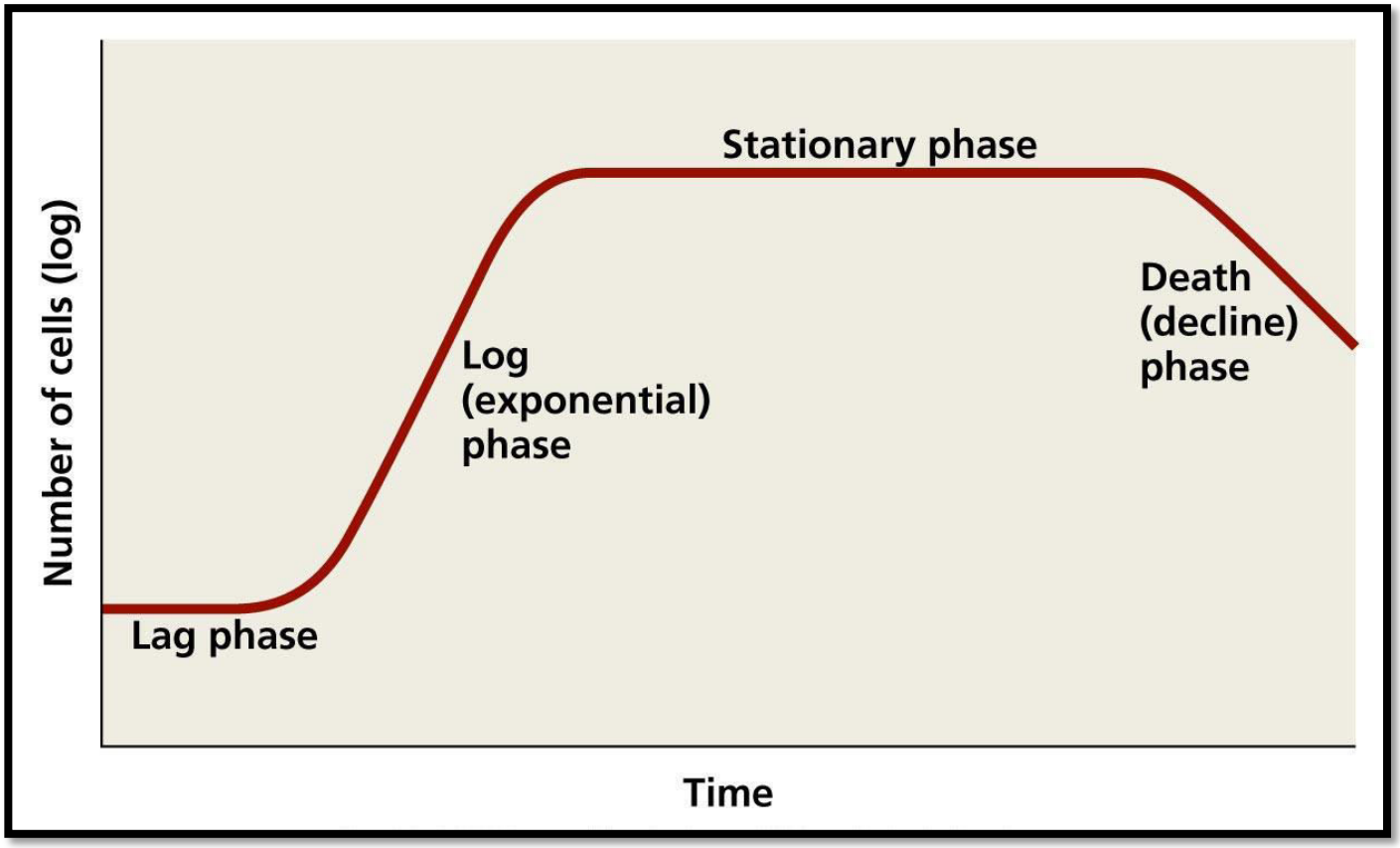
If a small number of an organism is placed in a suitable fluid nutrient medium under appropriate physical and chemical conditions, then the number of viable cells per milliliter is determined periodically, and plotted, a characteristic growth curve with four phases is obtained.

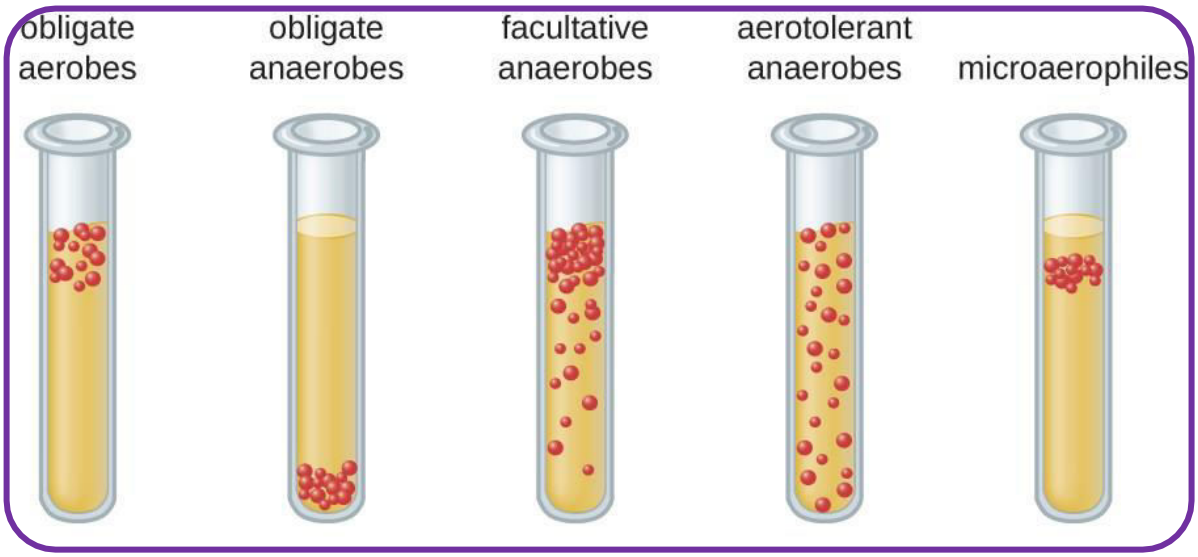
1. **Lag phase:**
- The initial number of bacterial cells remains constant.
- During this period, **the cells adapt** to their new environment.
- Enzymes and intermediates are formed to permit growth.

2. **Exponential (logarithmic) phase**:
- There is **marked increase** in cell number and its rate is accelerated exponentially with time giving a characteristic linear plot on a logarithmic scale.
- In this phase, the organism shows typical morphology.

3. **Stationary phase:**
   - ❖ Exhaustion of nutrients and accumulation of toxic products cause growth to decrease.
   - ❖ There is slow loss of cells through death which is just balanced by formation of new cells through growth and division.
   - ❖ **The number of viable bacteria remains constant.**

4. **Decline phase:**
   - ❖ At the end of the stationary phase, the death rate increases and exceeds the multiplication rate due to nutrient exhaustion and accumulation of toxic metabolic end products.
   - ❖ **So, the number of viable bacteria decreases.**





MCQs

1. **What types of bacteria synthesize organic compounds from Inorganic compounds?**
   a- Heterotrophs
   b- Obligate anaerobes
   c- Aerobes
   d- Facultative anaerobes
   e- Autotrophs

2. **Which of the following terms best describes bacteria that lack catalase but not superoxide dismutase?**
   a- Obligate aerobe
   b- Obligate anaerobe
   c- Facultative anaerobe
   d- Aerotolerant anaerobe
   e- Microaerophilic

3. **Capnophilic bacteria require:**
   a- Low concentration of O_2
   b- High concentration of O_2
   c- High concentration of CO_2
   d- Alkaline pH
   e- High temperature

4. **What type of bacterium is most likely to cause spoilage of refrigerated foods?**
   a- Mesophilic
   b- Thermophilic
   c- Psychrophilic
   d- Capnophilic
   e- Microaerophilic

5- **Bacterial cell death is balanced by the formation of new cells in:**
   a- Lag phase
   b- Exponential phase
   c- Stationary phase
   d- Decline phase
   e- Log phase

Chapter 4

Bacterial Viruses (BACTERIOPHAGES)

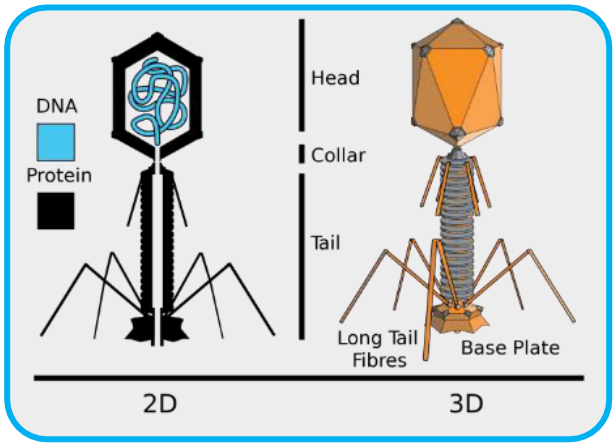
Definition:

Bacteriophages (or phages) are viruses that parasitize bacteria → the bacterial cell serves as a host for the virus.

Morphology of the Bacteriophage:

1. **A head:** containing the nucleic acid core (usually DNA, rarely RNA) surrounded by a protein coat (capsid).

2. **A tail:** consists of a hollow core surrounded by a contractile sheath which ends in a base plate to which tail fibers are attached.



Replication of Bacteriophages

Two cycles for phage replication are known:

A- Lytic (vegetative) cycle:

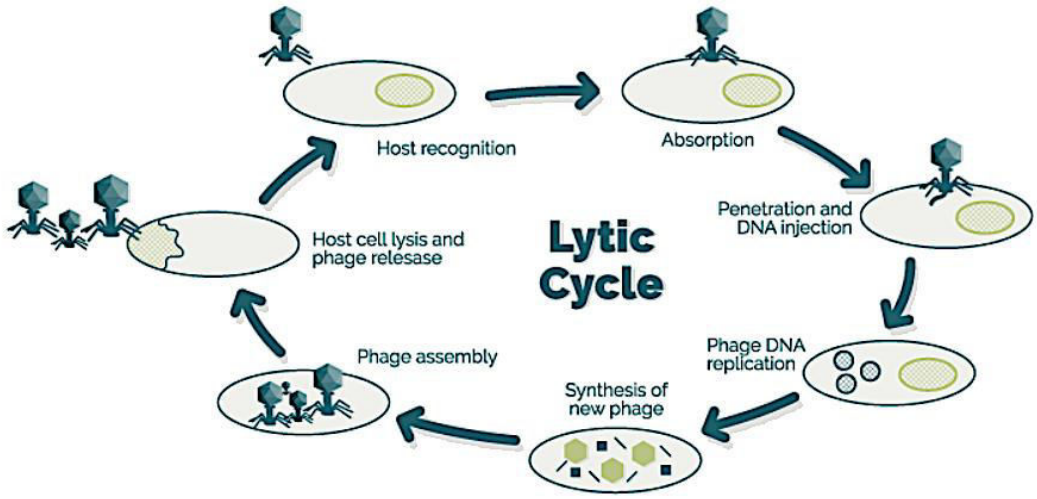
It is so-called because it ends in lysis of the bacterial host cell and release of the newly formed phages.

The stages of this cycle are:

1. **Adsorption:**
   - The phage attaches, by its tail, to specific receptors on bacterial cell.
   - The specificity of this process determines the susceptibility of bacteria to different phages.
2. **Penetration:**
   - The tail sheath contracts and the nucleic acid is injected into the cell.
   - The empty head and the tail are left outside the cell.
3. **Eclipse phase:**
   - in which no phage components are detected inside the cell.
   - It takes a short time (**minutes to hours**) during which viral nucleic acid directs the host cell metabolism to synthesize the enzymes and proteins required for phage synthesis.

4. **Intracellular synthesis of phage nucleic acids, capsids and tails.**
   - Several hundreds of phage components are synthesized.
5. **Assembly:**
   - The phage components aggregate to form complete phage particles which mature into typical infectious phages.
6. **Release:**
   - The bacterial cell bursts liberating a large number of phage particles to infect new cells.

During the lytic phage cycle, fragments of the bacterial DNA may be incorporated into the phage head. The phage can then transfer the incorporated bacterial DNA into another bacterial host "generalized transduction"



B - Temperate (lysogenic) cycle

In this cycle, the phage (temperate phage does not replicate and lyse the bacteria but the phage DNA becomes integrated with the bacterial chromosome and divides with it to pass into daughter cells.
The integrated phage genome is called "prophage" and the bacteria carrying it are called "lysogenic" bacteria

The presence of the prophage in the bacterium renders it:

1. **Immune to infection by another phage.**
2. **Lysogenic**: the bacterium acquires new properties → **diphtheria bacilli** can produce toxin only when lysogenized.
   Acquisition of a new character coded for by a prophage DNA is called "**lysogenic conversion**", or "**phage conversion**".
   When the phage is lost from the bacterium, this new characteristic is lost.

Outcome of the Temperate cycle

1. The prophage may be carried inside the bacterial cell indefinitely passing to daughter cells.
2. The prophage may be induced to detach from the bacterial chromosome and start a Lytic cycle. Induction may be spontaneous or achieved by an inducer as **U.V. light**.

During the process of induction, the prophage may carry with it few genes of the bacterial chromosome. When it infects another bacterium, it passes this fragment to it giving it new characters. This is known as "specialized transduction"

Practical Uses of Bacteriophages

1. **Phages are used as cloning vectors** in recombinant DNA technology. They carry and introduce foreign DNA fragments into a host cell.
2. **Phage typing**:
   - Since bacteria differs in their sensitivity to different phages, phages are used to identify and type strains of bacteria that are biochemically and antigenically indistinguishable.
   - This phage typing is important in epidemiologic studies → to trace the source of infection in outbreaks of post-operative wound sepsis caused by ***Staphylococcus aureus.***
3. **Phages are used as research elements** in some biological and genetic studies.

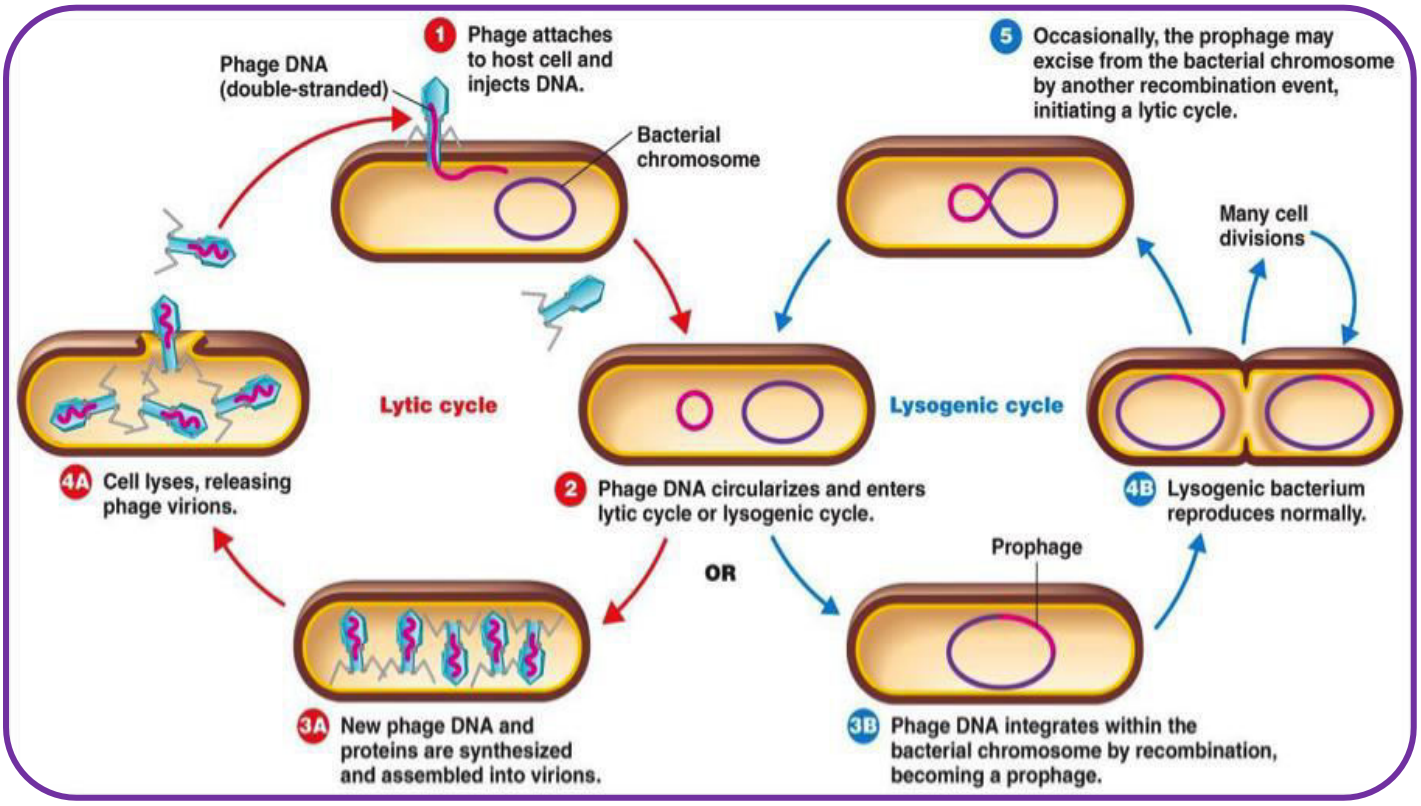
MCQs

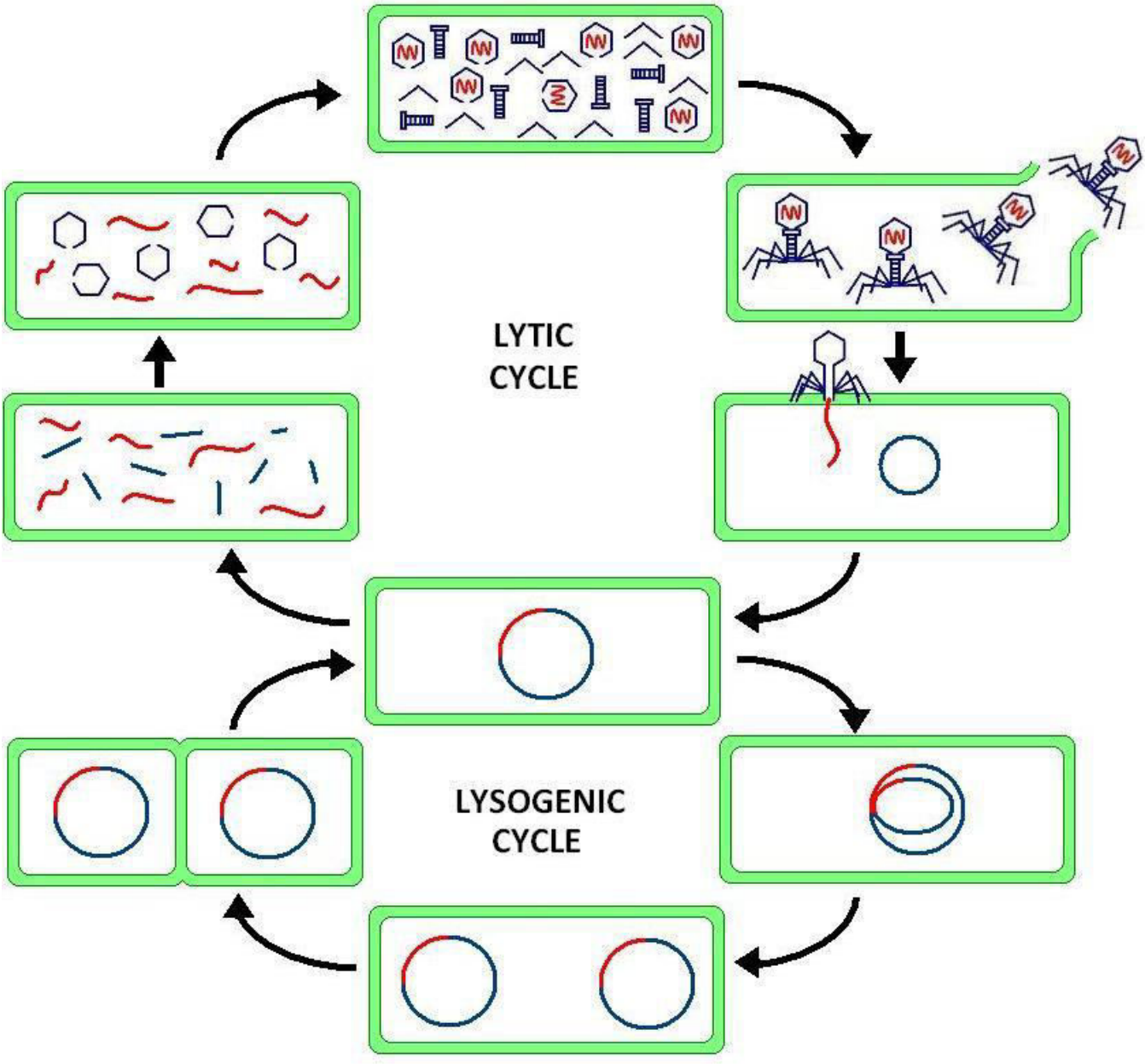
1- In lytic cycle of bacteriophages all the following occur EXCEPT:

a- Lysis of the bacterial host cell & release of newly formed phages.
b- The tail sheath contracts & nucleic acid is injected into the cell.
c- The phage attaches by its tail to a specific receptor.
d- The prophage is carried inside the bacterial cell indefinitely passing to daughter cells.
e- The phage components aggregate to form complete phage particles.

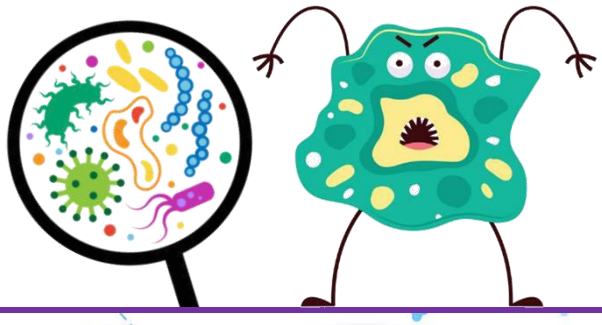
2- The lysogenic bacterial cell Is the cell containing:

a- Lysosomes
b- Lysozymes
c- Bacteriocins
d- Prophage
e- Endospores

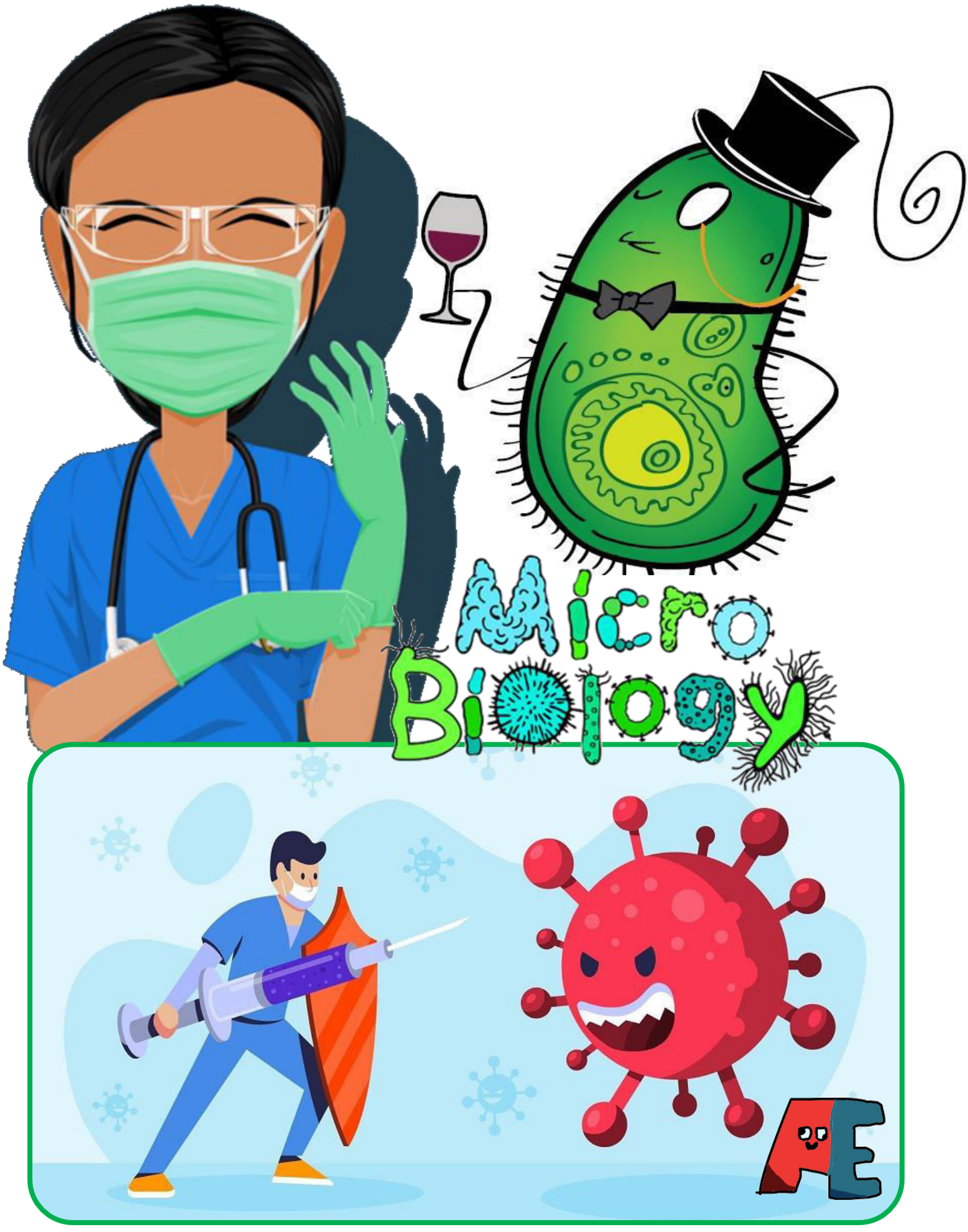

1 Phage attaches to host cell and injects DNA.
Phage DNA (double-stranded)
Bacterial chromosome
5 Occasionally, the prophage may excise from the bacterial chromosome by another recombination event, initiating a lytic cycle.
Many cell divisions
Lytic cycle
Lysogenic cycle
4A Cell lyses, releasing phage virions.
2 Phage DNA circularizes and enters lytic cycle or lysogenic cycle.
4B Lysogenic bacterium reproduces normally.
OR
Prophage
3A New phage DNA and proteins are synthesized and assembled into virions.
3B Phage DNA integrates within the bacterial chromosome by recombination, becoming a prophage.


LYTIC CYCLE
LYSOGENIC CYCLE

General MICROBIOLOGY



PART 2


Micro
Biology

Chapter 5

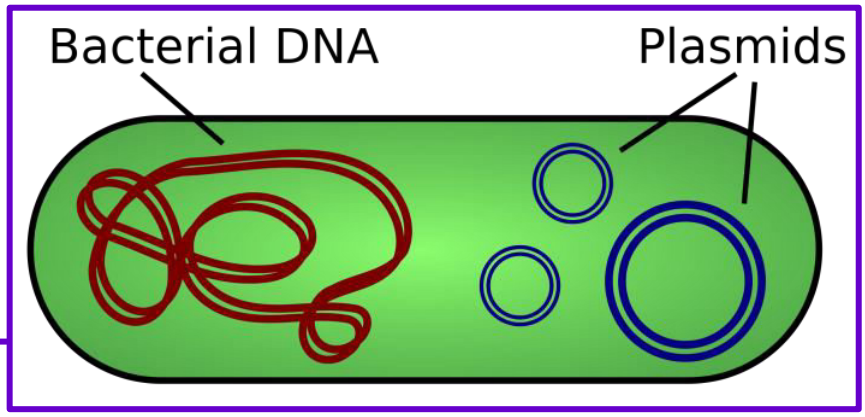
BACTERIAL GENETICS

- Genetics is the science which defines and analyzes heredity.
- The unit of heredity is the gene.
- **The Gene:** a segment of DNA that carries information for a specific biochemical or physiologic property.

The bacterial genome: the total set of genes present inside the bacterial cell.

It comprises:

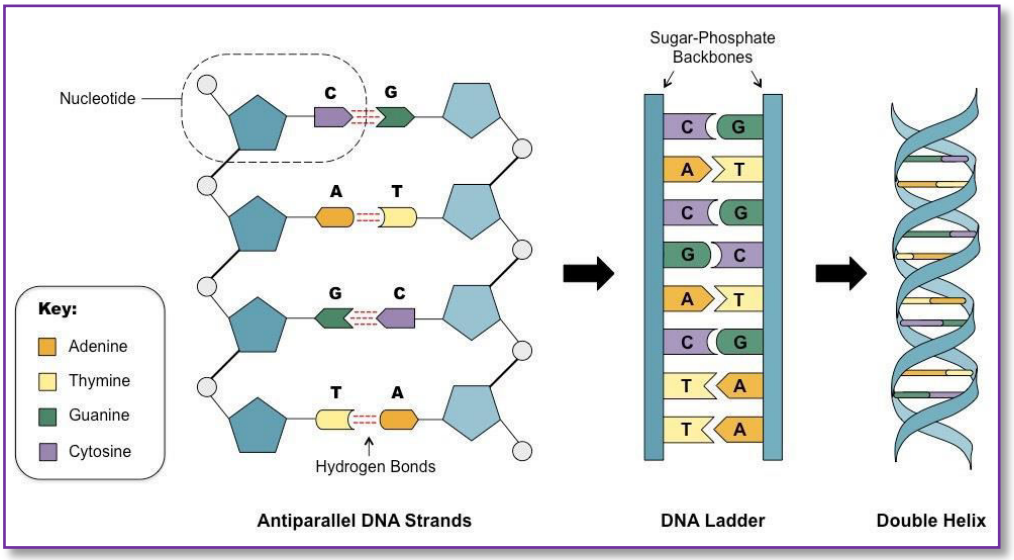
1. **The bacterial chromosome**: that can encode up to **4000** separate genes necessary for bacterial growth and propagation.
2. **Plasmids**
3. **Transposable genetic elements**
4. **Bacteriophage DNA** (**prophage**).



1. The Bacterial Chromosome:

- **Being a prokaryote** → the bacterial cell lacks a nuclear membrane.
- The DNA is concentrated m the cytoplasm as a nucleoid.
- **The nucleoid** consists of a single chromosome, which is a circular, supercoiled, double-stranded DNA molecule, associated at one point with a **mesosome**.
- This attachment plays a role in the separation of the two sister chromosomes following chromosomal replication.

- **The bacterial chromosome has the general chemical structure of DNA molecules**:
  - Each strand is formed of regularly alternating phosphate and sugar (**deoxyribose**) groups.
  - A nitrogenous base (**A**, **G**, **C**, or **T**) is attached to the sugar group and is projecting inwards towards the other strand.
  - **The two strands** are held together by hydrogen bonds between complementary bases (**A-T**) or (**C-G**) present at the same level.
  - The average length of the bacterial chromosome is **4000-5000 Kbps**.



The bacterial chromosome replicates by the semi-conservative method of DNA replication →

- The two strands are separated.
- **Each strand** acts as a template to synthesize a complementary strand through the action of the polymerase enzyme.
- **The bacterial chromosome** follows the same rules of gene expression and protein synthesis (transcription and translation) as higher organisms.

2. Plasmids

- **Plasmids are** → extra-chromosomal, circular, double-stranded DNA molecules dispersed in the cytoplasm.

- They are much smaller than the bacterial chromosome (**from several to 100 Kbps**).
- Plasmids are capable of replicating independently of the bacterial chromosome.
- Multiple copies of the same plasmid may exist in the same cell (**plasmid copy number**).

According to the copy number, plasmids can be categorized into 2 groups:

1. **Relaxed replicating plasmids:**
   - They can replicate in the absence of protein synthesis.
   - They are usually present in **30-50 copies/cell**, and are relatively small in size.

2. **Stringent plasmids:**
   - They require protein synthesis.
   - They are usually large and present in a **few copies (1-5) per cell**.

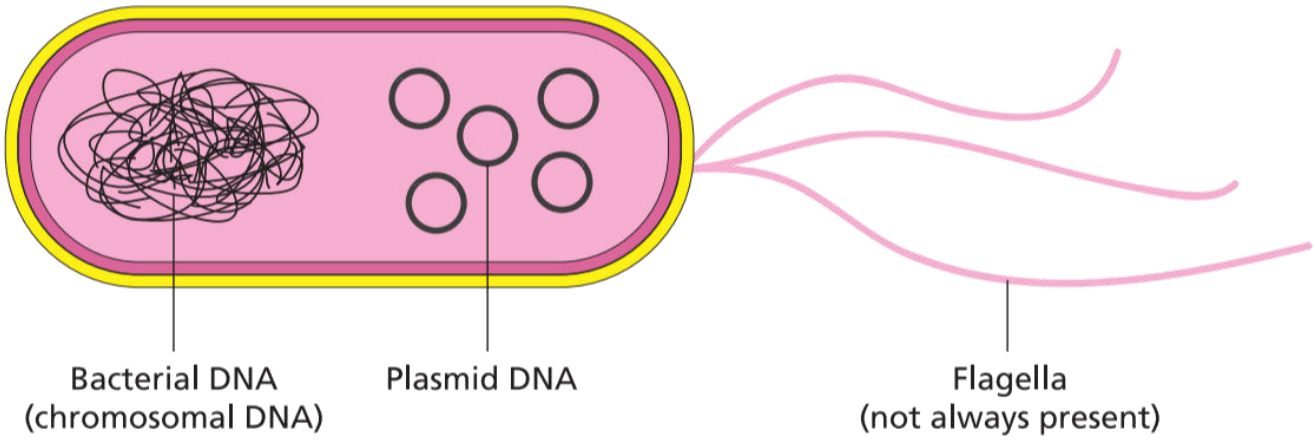
Plasmids are generally dispensable →

- This indicates that most plasmids encode properties that are not essential for growth, replication or survival of the host bacterium.

This is evidenced by:

a- The spontaneous loss of plasmids during cell division.

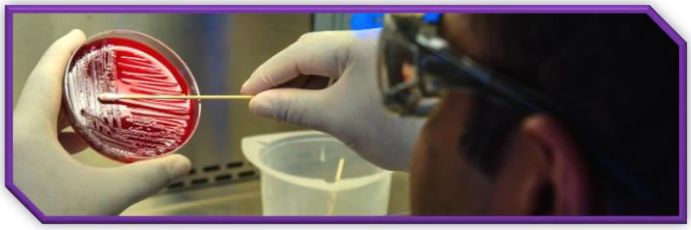
b- **Plasmid curing**: The experimental kicking off of the plasmids using physical agents (**heat**) or chemical agents (**antibiotics**).



Functions (traits) exhibited by plasmids:

1. **Sex pilus formation:**
   - Some plasmids carry fertility (**F**) factors that code for the formation of a sex pilus which mediates the process of conjugation.
   - such plasmids are also known as conjugative plasmids.

	Conjugative plasmids	Non-conjugative plasmids
Size	Large	small
Copy number	**1-2** (**stringent**)	**>30** (**relaxed**)
F factors	Present	Absent
Sex pilus formation	Yes	No
Transfer	By conjugation	With the help of conjugative plasmid
Host bacteria	Common in Gram **-ve bacilli**	Common in Gram **+ve cocci**



2. Antibiotic resistance:

- Some plasmids carry genes for resistance (**R-factors**) to one or several antimicrobial drugs.
- They often control the formation of enzymes capable of destroying the antimicrobial drugs.
- **Example**: **β-lactamase** which determines resistance to penicillin and cephalosporins.
- R-factors are usually conjugative plasmids that can be transferred among bacteria by conjugation.
- **This results in the rapid spread of drug-resistance** among bacterial populations and the development of multiple drug-resistant bacterial strains.

3. Virulence plasmids:

- **may code for:**
  - exotoxins.
  - Adhesins.
  - invasion factors.

4. Bacteriocin production:

- **Bacteriocins**: bactericidal substances produced by certain bacterial strains and are active against other strains of the same or closely related species.

- **Example** → **colicin E1** produced by **E.coli**.

5. Other functions:

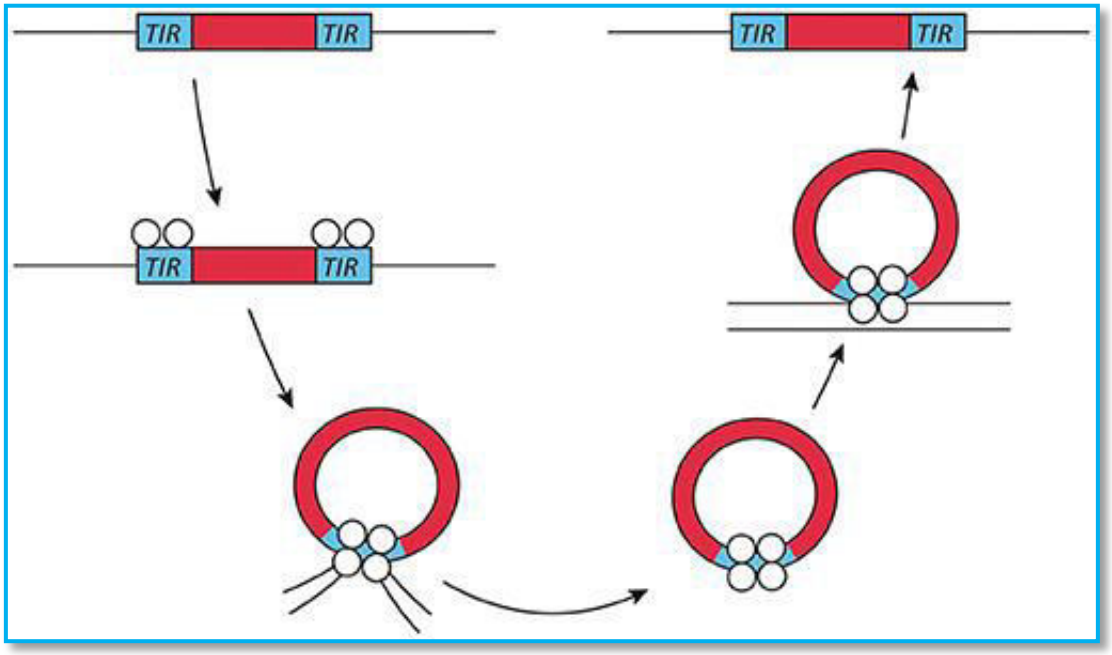
a- Nitrogen fixation.
b- Sugar fermentation.
c- Antibiotic production.
d- H_2S Production.
e- Resistance to heavy metals.
f- Degradation of aromatic compounds.

3. Transposable Genetic Elements

- **Definition**: non-replicating DNA segments that are capable of inserting themselves into other DNA molecules.
  They are also capable of mediating their own transfer from one location to another on the same chromosome or between chromosomes and plasmids.

- **Transposition** occurs infrequently (**once every 10^5-10^7 generations**) often in a random pattern.
- The insertion of a transposable element into a gene usually leads to inactivation of that gene.

- **Classes**:
  a- **Transposons** → which encode specific genes (**such as** antibiotic resistance).
  b- **Pathogenicity islands (PAI)** → which give the bacterium a variety of virulence characters → **such as** the ability to adhere to or invade host cells.



3. Bacteriophage DNA

The DNA of the temperate bacteriophage that is integrated in the chromosome of a lysogenic bacterial cell (**the prophage**) is considered as a part of the genome of such bacteria.

MCQs

1- Bacterial genetic information is carried on the following EXCEPT:

a. Ribosomes
b. Chromosome
c. Transposons
d. Plasmids
e. Bacteriophage

2- Plasmids:

a) Are single-stranded DNA molecules.
b) Carry optional genes (are dispensable).
c) Carry genes essential for growth
d) Are always found in linear form
e) Are always present as one copy/cell

3- Plasmids differ from transposable genetic elements, as plasmids:

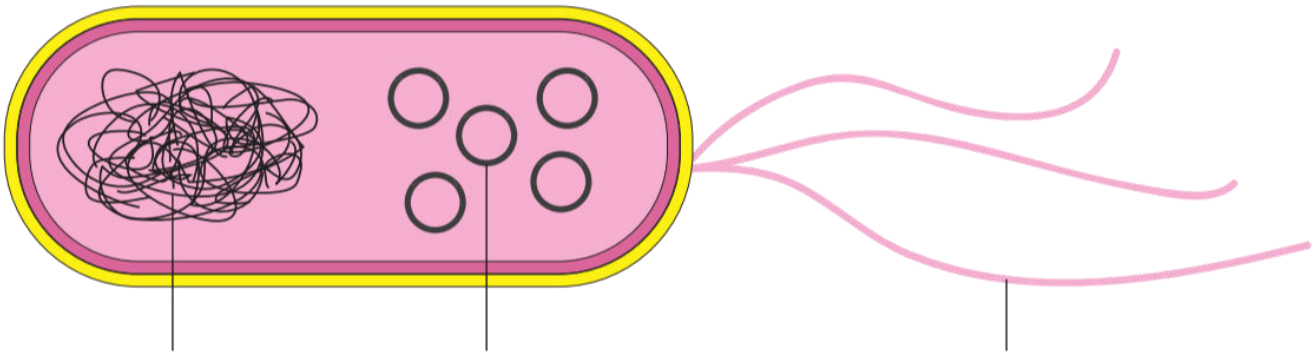
a) Become inserted into chromosomes
b) Are self-replicating outside the chromosome
c) Move from chromosome to chromosome
d) Carry genes for virulence (exotoxin production)
e) Carry genes for antibiotic resistance

4- Plasmids may code for any of the following EXCEPT:

a) Sex pilus formation
b) Bacteriocin production
c) Growth
d) Antibiotic resistance
e) Virulence

5- Conjugative plasmids:

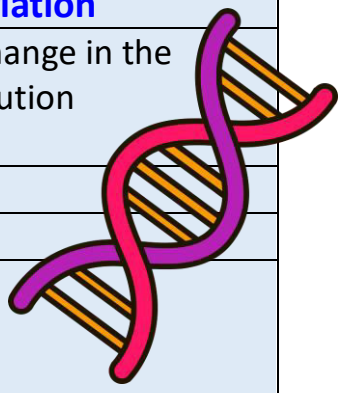
a) Are usually small in size
b) Carry fertility (F) factor
c) Are relaxed plasmids
d) Have a large copy number
e) Are common in Gram positive cocci



Chapter 6

BACTERIAL VARIATION

- **Bacterial variations** are changes in the bacterial characters.
- They may be phenotypic or genotypic.

Phenotypic variation	Genotypic variation
It occurs in response to changes in the environmental conditions without changes in the genetic constitution	It occurs as a result of a change in the underlying genetic constitution 
Reversible (**transient**)	Irreversible (**permanent**)
Not heritable	Heritable
Examples: 1- L-Forms. 2- Loss of flagella upon exposure to phenol. 	**Examples**: 1- Mutation 2- **Gene transfer**: a- Transformation b- Transduction c- Conjugation

MUTATION

It results from a change in the nucleotide sequence of DNA that may occur:

a) **Spontaneously** as a replication error (**rate of once every 10^6-10^7 cells**).
b) **induced** by radiation or chemical agents (**higher rate of once every 10^3-10^4 cells**).

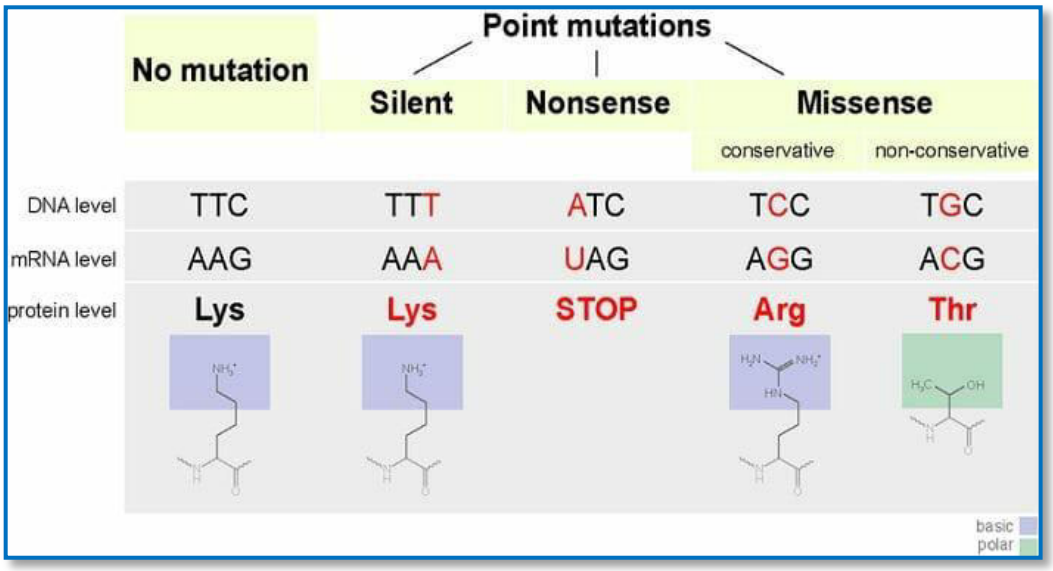
Mutation can be classified into:

1. **Single-base (point) mutations**:
involve the replacement (**substitution**) of a single nucleotide in the coding sequence.

This may result in:

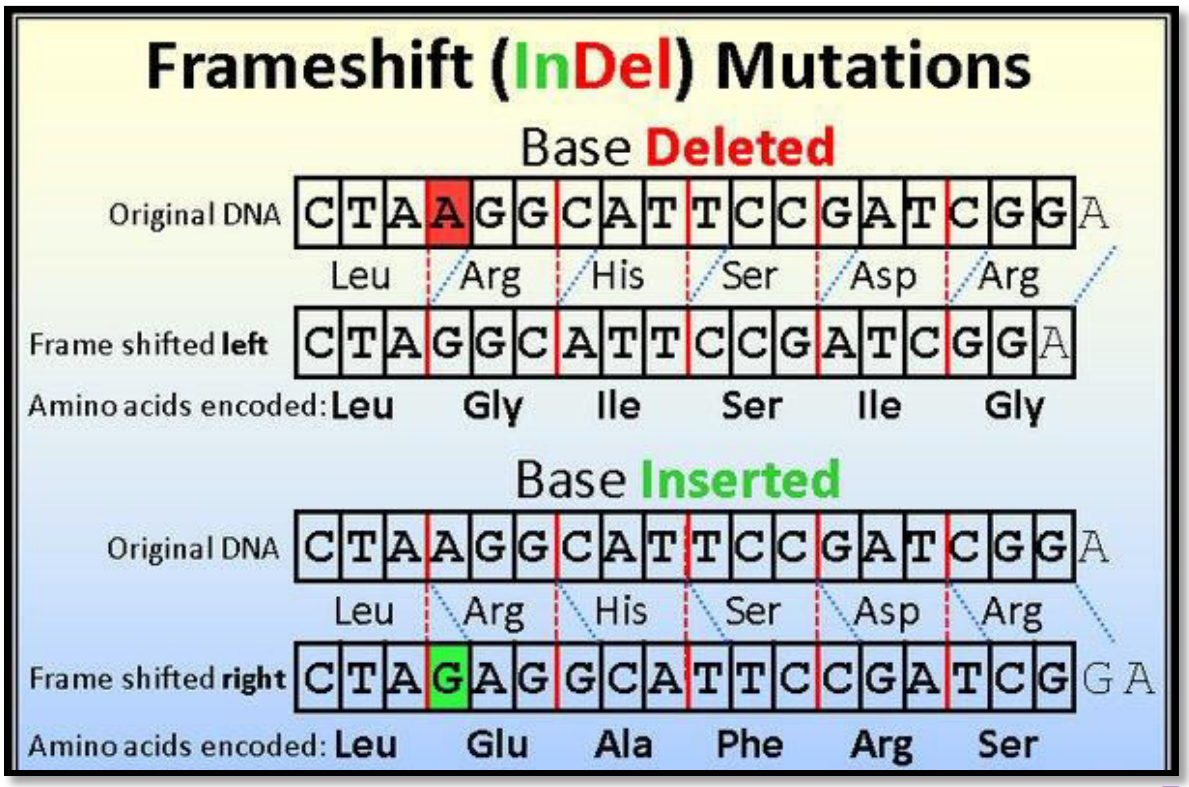
a- **Same sense (silent) mutations**: occur when the resulting base triplet (codon) codes for the same amino acid as the original triplet.
b- **Missense mutations**:
- occur when the mutant base changes the coding sequence so that a different amino acid is produced.

- The resulting protein may be functioning or not, depending on the importance of the area affected by the mutation.



2. **Frame-shift mutations**: occur when a nucleotide is inserted into, or deleted from the coding sequence, resulting in a shift of the reading frame → insertion of a transposable element.

Induced mutations may be used to manipulate viral genomes for vaccine production and gene therapy.

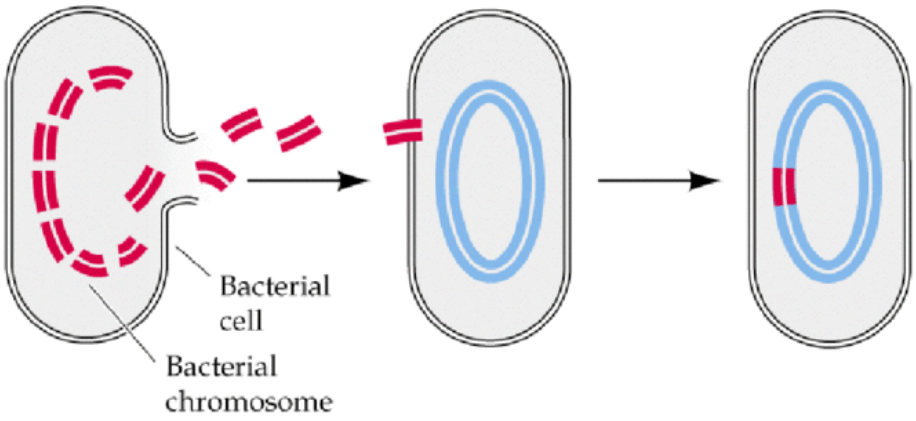


Gene Transfer

There are 3 methods for gene transfer among bacteria

1. Transformation

- Dying bacteria release DNA which can be taken up by other bacteria.
- Such DNA may be chromosomal or plasmid in origin, and may carry genes that "**transform**" the Recipient bacterium.
- **The transforming DNA** may become integrated with the bacterial chromosome or re-established extra-chromosomally in the recipient cell.
- **Transformation depends on competence**, which is the ability of the recipient bacterial cell to take up DNA.
- **Competence** depends on the presence of proteins in the cell membrane that have a special affinity to bind DNA and transport it into the cytoplasm.
- Artificial competence can be induced during recombinant DNA techniques by treating the recipient bacteria with calcium chloride, which alters cell membrane permeability, enabling the uptake of DNA.



2. Transduction

It is the transfer of DNA from one cell to another by means of a bacteriophage.

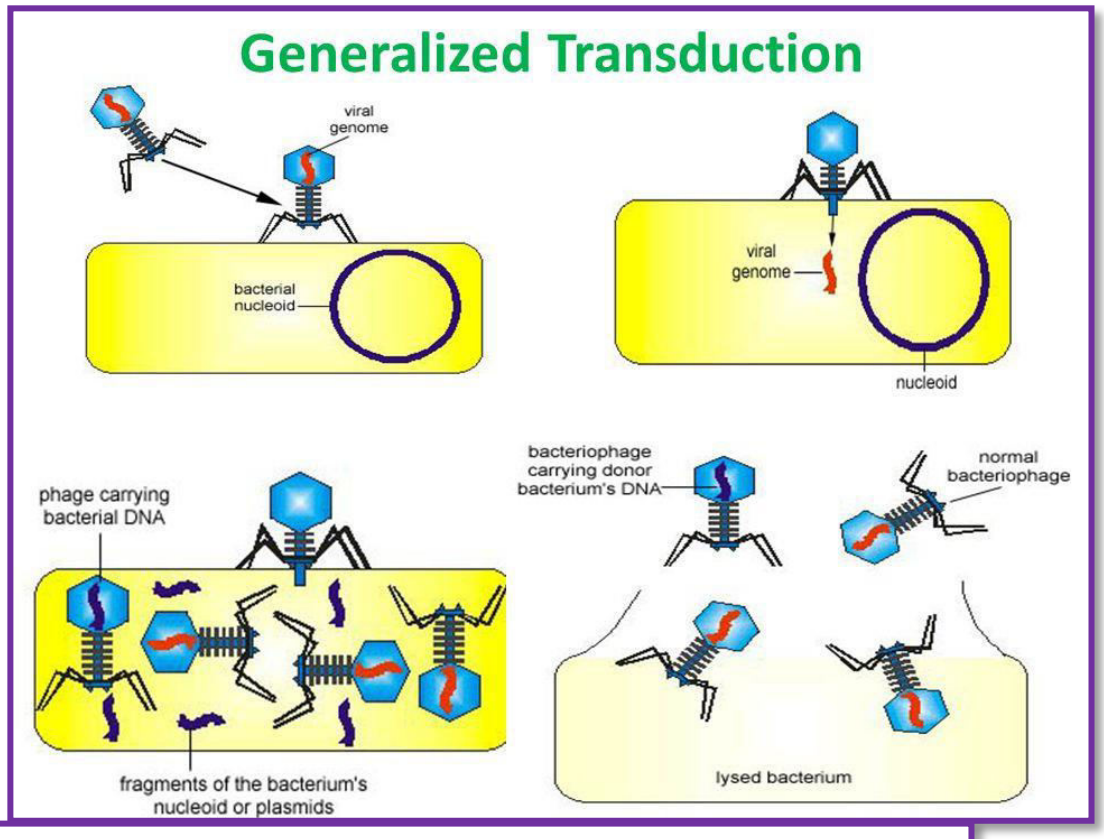
Types of Transduction:

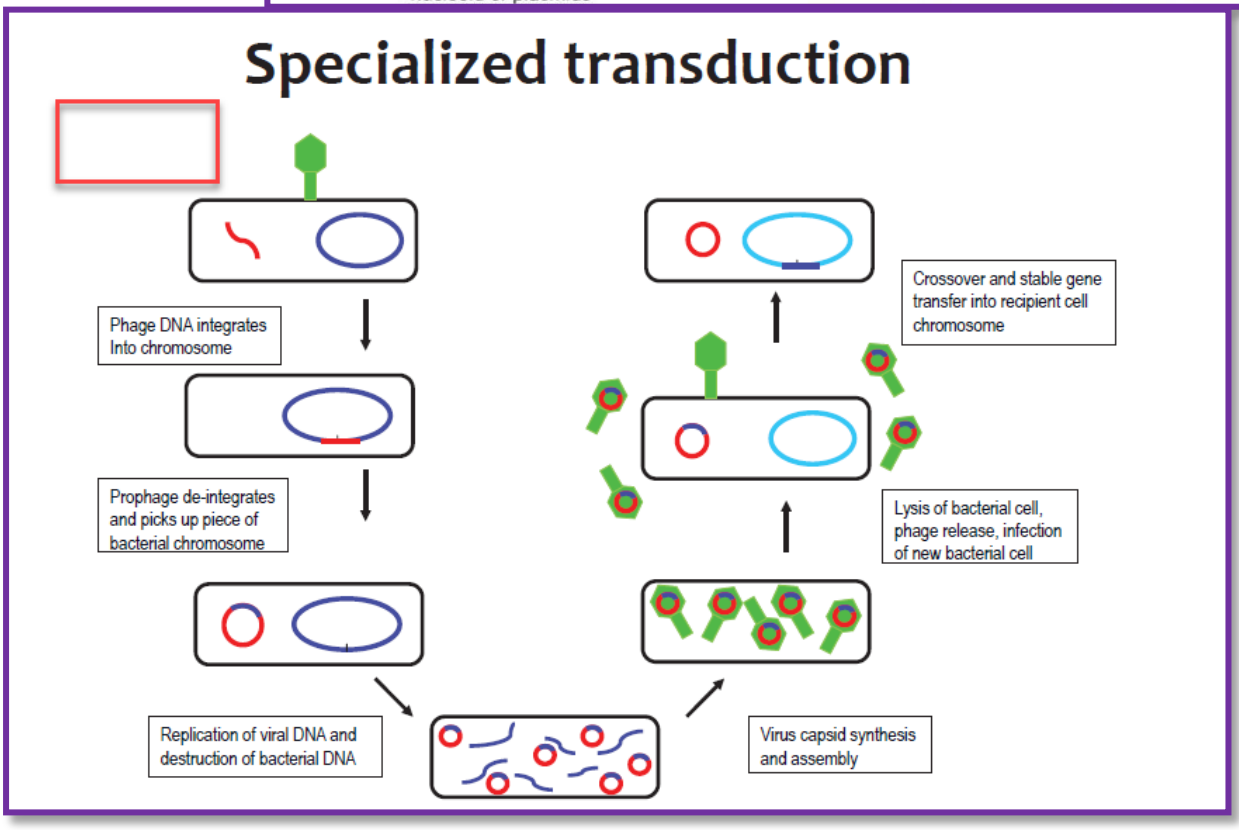
1) Generalized transduction:

- **During the lytic phage cycle**, the bacterial DNA is fragmented and any fragment of DNA (whether chromosomal or plasmid) may be incorporated into the phage head.
- The phage particle can then transfer the incorporated bacterial DNA into another bacterial host.

2) Specialized transduction:

- It takes place when a **prophage** contained in a lysogenized bacterial cell is induced to detach.
- **Such prophage** may carry with it the adjacent piece of the chromosomal DNA and transfer it to another bacterial cell.



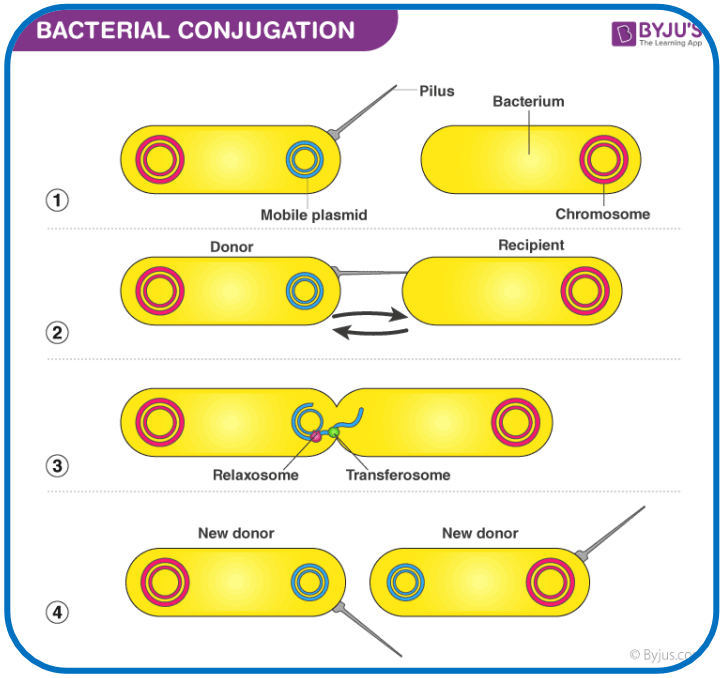


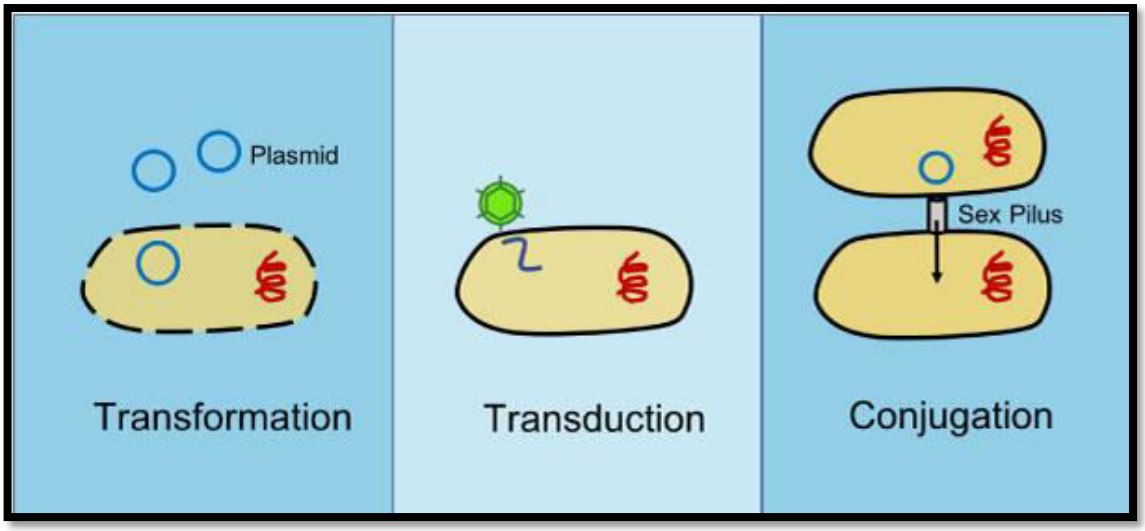
3. Conjugation:

- **It is the most frequently observed mechanism of DNA transfer.**

- **It involves 2 cell types**:
    1. **donors (F+)** which possess the fertility (F) factor
    2. **Recipients (F)** which lack the F factor.

- The F factor carries the genes for the synthesis of the sex pilus which acts as a conjugation tube between the donor and recipient bacterial cells.
- The 2 DNA strands of the **F factor** are then separated, and one strand is transferred from the donor to the recipient cell.
- Each strand forms a complementary strand.
- **Result**: the recipient cell acquires a copy of the F plasmid and becomes an **F+** cell.





MCQs

1- Transformation in bacteria depends on:

a. F factors
b. R factors
c. Bacteriophages
d. Cosmids
e. Competence of bacteria

2- One of the following requires cell to cell contact:

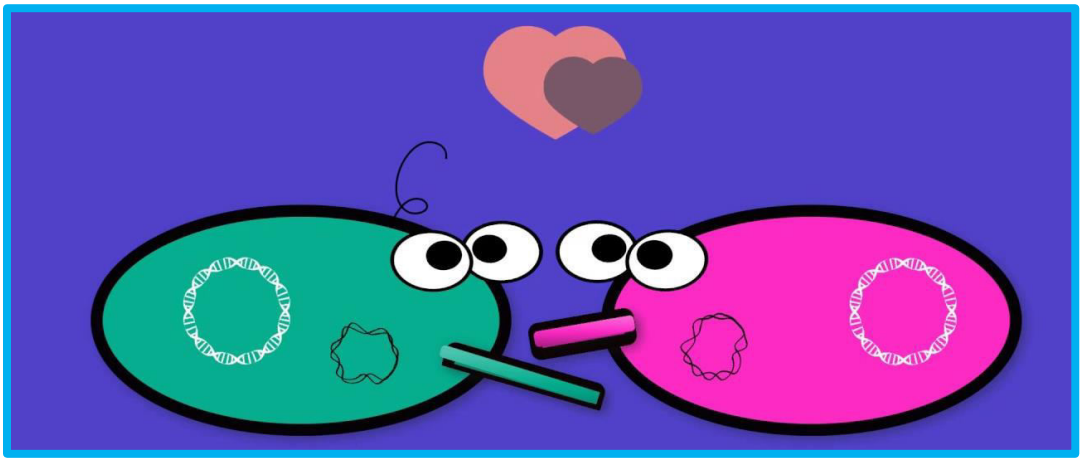
a- Transformation
b- Conjugation
c- Transduction
d- Transcription
e- Transposition

3- Which of the following is mediated by a bacteriophage that carries host cell DNA:

a) Transformation
b) Conjugation
c) Transduction
d) Translation
e) Transcription

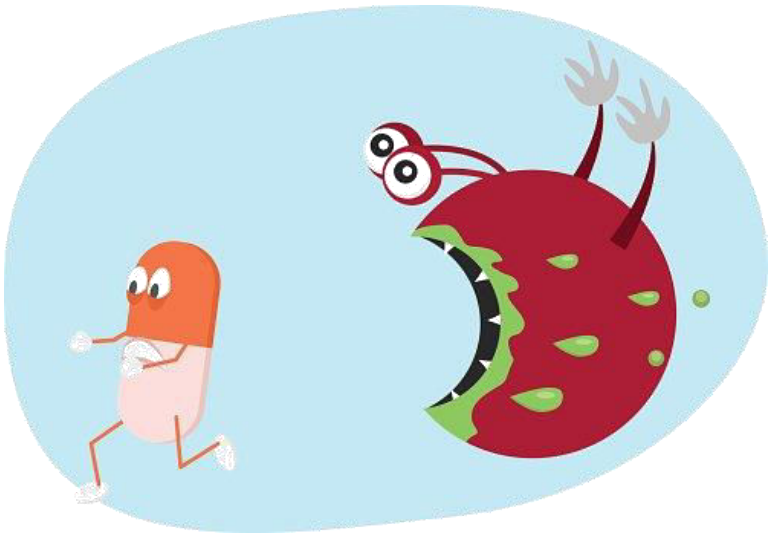
4- Regarding generalized transduction:

a) It occurs during lytic cycle of bacteriophage.
b) It occurs during lysogenic cycle of prophage.
c) A specific piece of bacterial DNA is transferred from one cell to another.
d) Sex pilus is necessary.
e) It results in phenotypic variation of bacterial character



Chapter 7

ANTIMICROBIAL CHEMOTHERAPY



Antimicrobial chemotherapeutic agents:

- are chemically synthesized substances that are used to treat infectious diseases by killing or inhibiting the growth (or multiplication) of microorganisms.

Antibiotics:

- are low-molecular weight antimicrobial substances that are produced as secondary metabolites by certain groups of microorganisms → ***Streptomyces***, ***Bacillus***, and a few molds (***Penicillium*** and ***Cephalosporium***).
- Although their original source was a microorganism → some antibiotics are currently made synthetically (synthetic antibiotics).
- Chemical modification of certain antibiotics, to achieve the desired properties, has been a prominent method of new drug development (semisynthetic antibiotics).

Bacteriostatic agent:

- is an antimicrobial agent that is capable of inhibiting bacterial multiplication.
- Multiplication resumes upon removal of the agent.

Bactericidal agent:

- is an antimicrobial agent that is capable of killing bacteria.
- Multiplication cannot be resumed.

Selective toxicity:

- **is the ability of an antimicrobial agent to harm a pathogen without harming the host.**
- It may be a function of a specific receptor (or target) for the drug found in the microbe but not in the human body (**peptidoglycan**), or it may depend on the inhibition of a biochemical event essential for the organism but not for the host.

Spectrum of activity:

- the range of microorganisms that are affected by certain antibiotics is expressed as its spectrum of action.
- Antibiotics which kill or inhibit the growth of **a wide range** of Gram-positive and Gram-negative bacteria are said to be **broad spectrum**.
- If effective mainly against **either** Gram-positive or Gram-negative bacteria, they are **narrow spectrum**.
- If effective against a **single organism** or disease, they are referred to as **limited spectrum.**

Mechanisms of Action of Antimicrobial Agents

1. Inhibition of bacterial cell wall synthesis

1. **β-lactam antibiotics** → **penicillin, cephalosporins**.
2. **Glycopeptides** → **vancomycin, teicoplanin**.
3. **Cycloserine and bacitracin**.

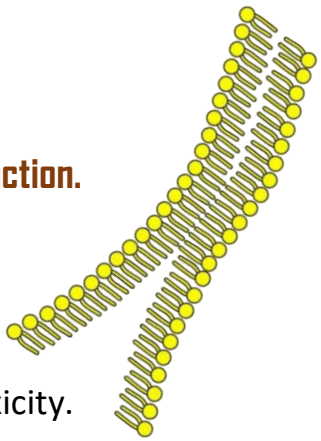
- **These antibiotics are bactericidal with minimal tissue toxicity.**

- **The β-lactam drugs** inhibit the last steps of peptidoglycan synthesis.
  This inhibition is initiated by binding of the drug to certain cell receptors known as penicillin-binding proteins (**PBPs**).
- **glycopeptides & cycloserine** inhibit early steps in the biosynthesis of peptidoglycan, which occur inside the cytoplasmic membrane.

- **The mechanism of resistance** to β-lactam antibiotics is different from that for the other groups.

- **vancomycin** could be used successfully in infections caused by β-lactam resistant staphylococci.

2. Interference with the cell membrane function



Some agents disrupt the cytoplasmic membrane and interfere with its function.

a- **Antibacterial agents**: **polymyxin** and **colistin**.
b- **Antifungal agents**: **Amphotericin B**, **nystatin** and **imidazoles**.
- These agents are **Microbicidal**.
- They are highly toxic as they have narrow margin of selective toxicity.

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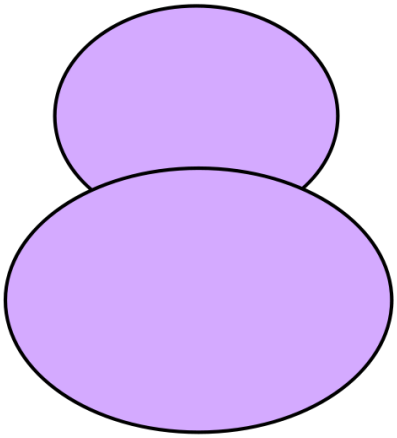
3. Inhibition of bacterial protein synthesis

- **Bacteria** have **70S** ribosomes (with **30S** and **50S** subunits) whereas **mammalian cells** have **80S** ribosomes (**40S** and **60S** subunits).
- **This difference makes bacterial ribosomes a selective target for antimicrobials**



1. **Agents acting on 30S ribosomal subunit**:
   tetracycline and **aminoglycosides**
   (**gentamicin**, **amikacin**, **streptomycin**).

2. **Agents acting on the 50S ribosomal subunit**:
   macrolides (**erythromycin**,**azithromycin**),
   lincomycins (**clindamycin**), **streptogramins**,
   linezolid, **chloramphenicol** and **fusidic acid**.

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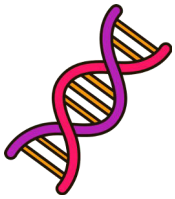
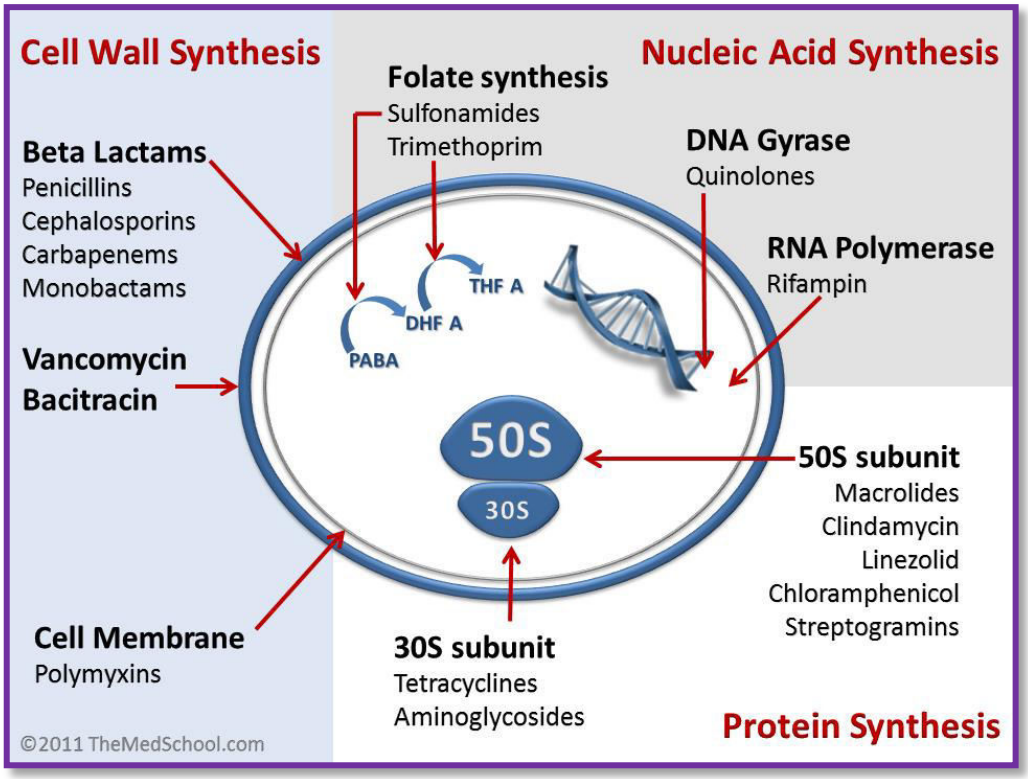
4. Inhibition of bacterial nucleic acid synthesis:



1. **Inhibition of RNA synthesis** through the strong binding to DNA-dependent RNA polymerase: **rifampin**.
2. **Inhibition of DNA synthesis** through blocking DNA gyrase: **quinolones** and **novobiocin**.
3. **Inhibition of dihydrofolic acid reductase** leading to inhibition of folic acid synthesis.
   The latter is important for purine synthesis → nucleic acid formation.
   Examples → **trimethoprim** and **pyrimethamine**.
4. **Inhibition of folic acid synthesis by competitive antagonism** → **sulphonamides**.
   For many organisms → para-amino benzoic acid (**PABA**) is essential for the synthesis of folic acid.

Sulphonamides are structural analogues of PABA.
They compete with PABA for the active center of the enzyme involved in folic acid synthesis.
As a result, nonfunctional analogues of folic acid are formed and nucleic acid synthesis is inhibited.



Choice of an Antimicrobial Agent for Therapy

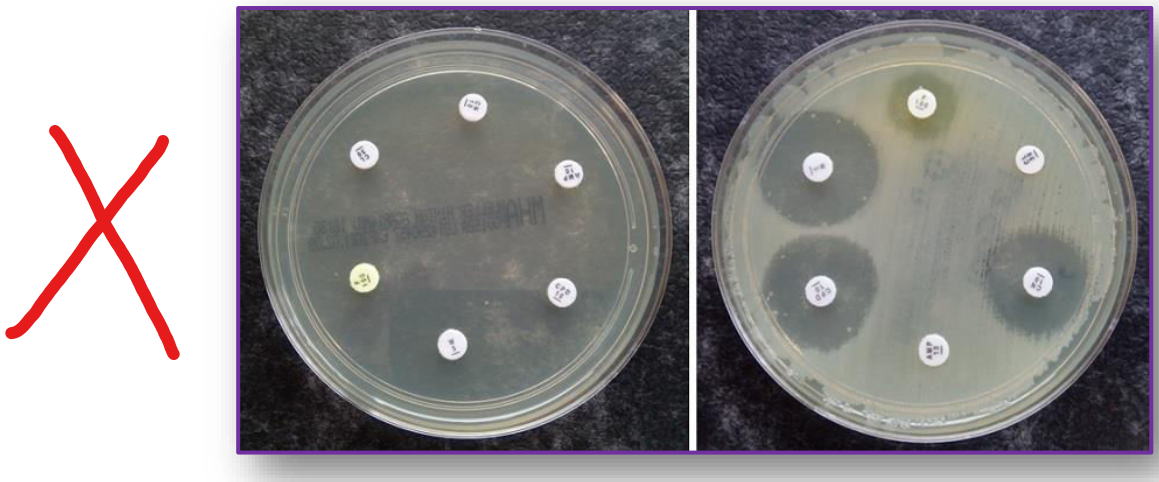
The following are guidelines that can be followed for proper antibiotic use:

1. **Select** an antibiotic that is able to penetrate to the site of infection and achieve effective concentration → certain drugs are able to pass the blood-brain barrier, others are highly concentrated in urine.
2. **Identify** the nature of the infection whether bacterial, viral, fungal, or parasitic.
   A common mistake is to give an antibacterial agent for a viral infection.
3. **Choose** as narrow an antibiotic spectrum as you can.
   When you get the results of culture and susceptibility, revise your treatment to narrow-down the spectrum as far as possible.
   The use of broad-spectrum antibiotics is likely to faster induce resistance to antibiotics and may be complicated by superinfection.

4. **Give the appropriate dose** of the antibiotic for the proper duration.
   Inadequate dosage or undue prolonged therapy may result in drug toxicity and antibiotic resistance.

5. **Know the potential of the drug to produce toxicity**: Some drugs known to be of low toxicity will exert high toxicity if they accumulate in the blood due to liver or kidney dysfunction. Use antibiotics that are only safe for the pregnant and lactating women and for infants and children.
6. **Choose bactericidal** rather than bacteriostatic antibiotics.

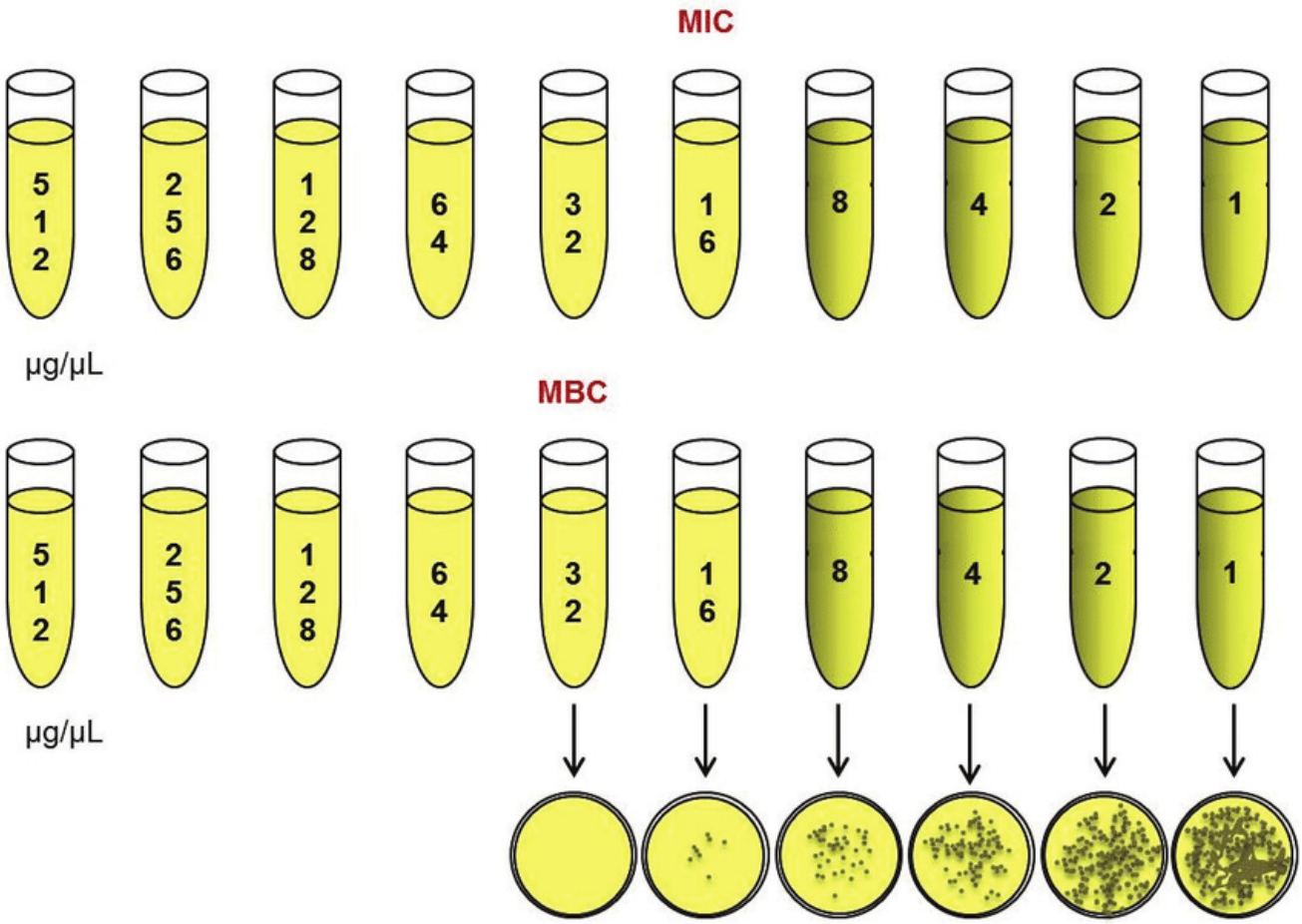
Microbial Susceptibilities to Antimicrobial Agents



- Microorganisms vary in their susceptibility to different chemotherapeutic agents.
- The appropriate antibiotic to treat any infection should be determined **in vitro** before any antibiotic is given.
- **The in vivo activity of an antimicrobial agent** is not always the same as its in vitro susceptibility because it involves many host factors that are not tested in vitro.
- The activity of an antimicrobial agent against an organism is dependent on its concentration.
- Some idea of the effectiveness of a chemotherapeutic agent can be obtained from determining the minimal inhibitory concentration (**MIC**).
- **The MIC** is defined as the lowest concentration of a drug that prevents growth of the test organism.
- **The MIC** forms the basis for susceptibility and determining breakpoints.
- The breakpoint of an antimicrobial agent is the concentration that can be achieved in the serum with optimal dose.
- Organisms with MICs at or below the breakpoint are considered susceptible.
- **On the other hand**, organisms with MICs above the breakpoint are considered resistant.

Routine in vitro susceptibility testing can be done by one of the following methods:

1. Disc diffusion method.
2. Dilution method such as tube broth dilution.
3. Gradient diffusion (**E test**) method.

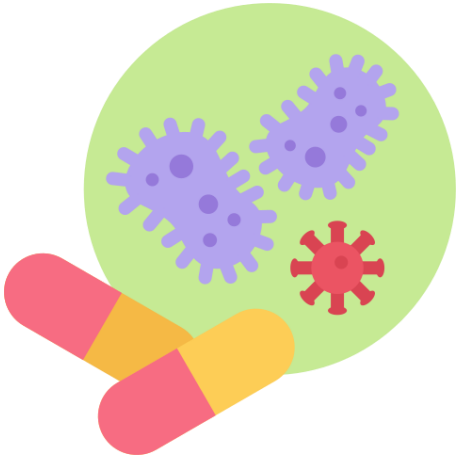


Empiric Therapy

- The empiric therapy is a "**best guess**" procedure based upon a provisional diagnosis made by the physician that a patient has a bacterial infection which requires treatment.
- **Depending on the type of infection**, there will be a short list of bacteria most likely to be causing that infection.
- **Depending on the type of bacteria**, there will be an antibiotic most likely to successfully treat that infection.
- "**Best guess**" treatment is not always successful or appropriate as many bacteria have unpredictable susceptibilities to antimicrobial agents.

Indications:

1. **In seriously-ill patients** empiric therapy should be started without delay but after collecting specimens for culture.
2. **In closed lesions**, where there is no available sample.



Combined Therapy

- The ideal rule in antimicrobial therapy is mono-therapy which means choosing one drug effective against a particular organism.
- There are conditions which necessitate the use of more than one antibiotic in order to achieve a successful clinical response.

Possible indications:

1- **Severe ill patients suspected of having serious infections:**
   a- Bacterial meningitis
   b- Sepsis in immunocompromised patients caused by **pseudomonas aeruginosa**, **klebsiella**, **Enterobacter**, **staphylococcus aureus**.
2- **Febrile neutropenia.**
3- **To delay the emergence of drug-resistant mutants** → treatment of **TB**.
4- **To achieve bactericidal action through synergistic effect** → in **enterococcal endocarditis.**
5- **Mixed infections** → infection following massive trauma.

Effects of combined therapy

1. Synergistic effect (1+1= >2):

- The combined action is significantly greater than the sum of both effects.
- Examples:
  1. **Vancomycin** + **gentamicin** in treatment of methicillin-resistant staphylococci.
  2. **Sulfamethoxazole** + **trimethoprim** (cotrimoxazole) in treatment of shigellosis.

2. Antagonistic effect (1 +1 =<1):

- The combined action is less than that of the more effective agent when used alone.
- Example:
  Penicillin + **chloramphenicol** in treatment of meningitis.

3. Indifference (1 +1 =1):

- The combined action is no greater than that of the more effective agent when used alone.
- Example:
  Cefepime + **vancomycin** …. **clindamycin** + **vancomycin**.

4. Addition (1 +1 =2):

- The combined action is equivalent to the sum of the actions of each drug when used alone.

Complications of Chemotherapy

1) **Toxicity:** may be dose-dependent or independent.

Examples:

a- **Tetracycline** may cause staining of teeth in infants.

b- **Streptomycin** may affect the 8th cranial nerve leading to vestibular dysfunction.

c- **Aminoglycosides** may cause nephrotoxicity.

d- **Chloramphenicol** can cause bone marrow depression.

2) **Allergy (hypersensitivity):** usually not dose-dependent.

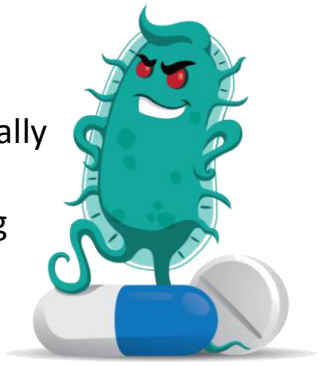
Examples:

a- **Penicillin** may cause urticaria, anaphylactic shock or serum sickness.

b- Local application of **sulphonamides** may result in contact dermatitis.

3) Emergence of resistant strains:

- **The abuse of antibiotics** (low dosage, interrupted course, no real indication, and improper choice) encourages the emergence of resistant mutants.
- These mutants will overgrow and replace the originally susceptible bacteria.
- It is recommended that in vitro susceptibility testing should be performed to guide the selection of antibacterial drugs.



4) Superinfection:

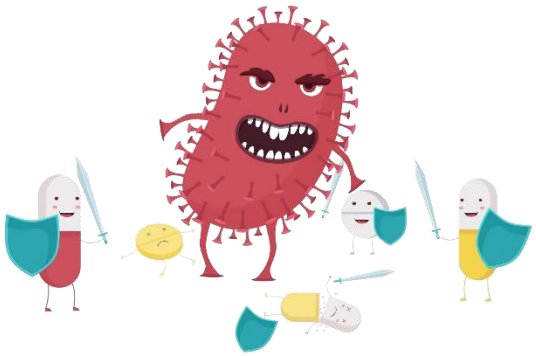
It occurs as a result of outgrowth of resistant members of normal flora when the sensitive ones are eradicated during antibiotic therapy.

Examples:

a- **Pseudomembranous colitis** caused by outgrowth of **Clostridium difficile**.

b- **Oral thrush** caused by overgrowth of the **yeast Candida**

Resistance to Antimicrobial Agents



- **Antibiotic resistance** is a global problem faced today in the treatment of infectious diseases.
- Resistance to antibiotics is more prevalent in hospitals especially intensive care units due to the higher antibiotic use.
- **Resistance to antimicrobial agents is of two categories either intrinsic or acquired.**

Intrinsic (inherent or natural) resistance

- This type of resistance refers to bacteria that are insensitive, in their natural state, to an antibiotic without the acquisition of resistance factors.
- It is consistent and can be expected once the organism is known.
- **Intrinsic resistance occurs in the following situations:**
  1. **Streptomycetes** are protected from the antibiotics they produce.
  2. **Gram-negative cell membrane** has pores too small to allow large antibiotic molecules, → **nafcillin**, to penetrate.
  3. **An organism lacks the target or receptor** for the antibiotic as in the case of resistance of Enterococcus species to cephalosporins.

Acquired resistance

- **It results from** altered bacterial physiology and structure due to changes in the genome of the organism.
- It is inconsistent and unpredictable.

- The unpredictable nature of this resistance is the 1ry reason why laboratory methods to detect resistance are necessary.

Acquired resistance mechanisms are driven by two genetic processes in bacteria:

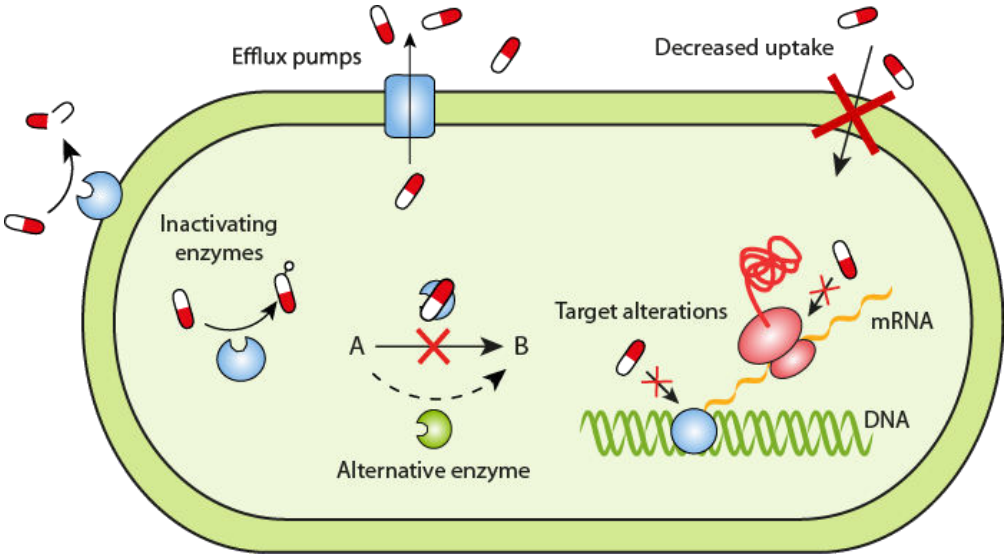
(1) **Mutation and selection** (sometimes referred to as vertical evolution): Exposure of an organism to an antibiotic exerts a selective pressure on the organism and leads to mutation.

The more frequent the exposure to the antibiotic the greater the potential resistance.

(2) **Exchange of genes between strains and species** (sometimes called horizontal evolution).

Resistance genes can be encoded on plasmids, phages and transposable genetic elements.

Mechanisms of acquired resistance



Bacteria have the ability to use one or more of the following mechanisms:

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A) Reduction of the intracellular concentration of the antibiotic by:

a) Decrease in influx of antibiotic through:

- Reduction of permeability of the outer membrane by modification or
- Loss of porin (a hollow membrane protein) required for entry of the antibiotic molecules.

b) Efflux pumps:

The antibiotic is pumped out across the cytoplasmic membrane faster than it can diffuse in, so the concentration of antibiotic remains too low to be effective.

B) Inactivation of the antibiotic:

- Production of **β-lactamases** leads to hydrolysis of the β-lactam ring → inactivating penicillin and cephalosporins.
- Production of acetyl transferase results in chloramphenicol resistance.
- Production of aminoglycosides-modifying enzymes

C) Target modification:

Modification of the target site for the antibiotic results in a reduced affinity for its receptor:

- Modification of the penicillin-binding proteins (**PBPs**) is a primary mode of resistance to β-lactam antibiotics in methicillin-resistant S. aureus (**MRSA**).
- alteration of the **50S** ribosomal subunit reduces the affinity of macrolides linezolid and streptogramins for the ribosome.
- Alteration of the **30S** ribosomal subunit reduces the affinity of aminoglycosides for the ribosome.

D) Target elimination by developing new metabolic pathways:

These bacteria have the ability to create new metabolic pathways that bypass the original target → resistance to trimethoprim.

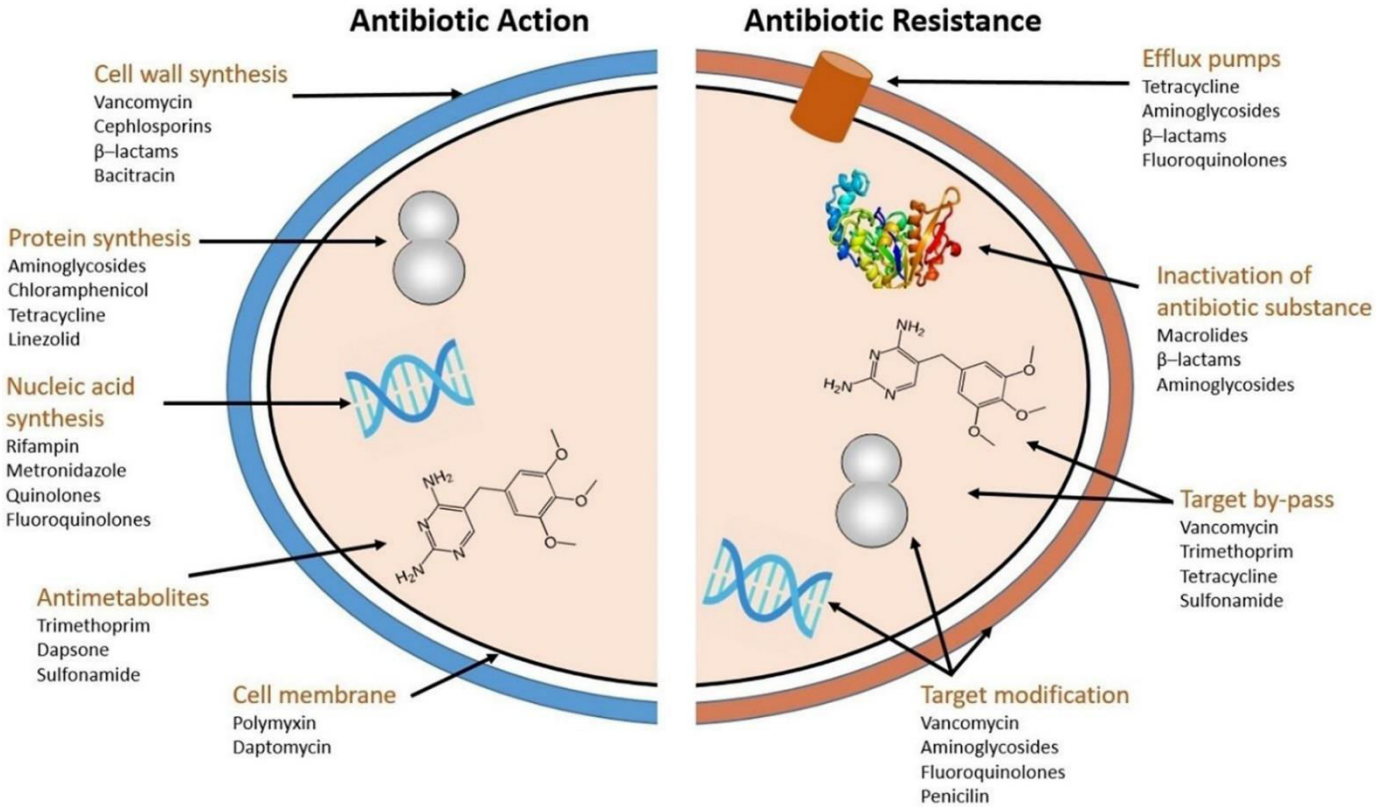
E) **Target overproduction:** This may be the mechanism used by S. aureus strains with intermediate susceptibility to vancomycin (**VISA**).

Antimicrobial Chemoprophylaxis

Chemoprophylaxis: the administration of an effective antimicrobial agent to prevent rather than to treat infection with a certain microbe, thus preventing development of a disease.

Examples:

1) **Long-acting penicillin (or erythromycin):** is given to rheumatic patients to prevent reinfection with S. pyogenes.
2) **Rifampicin:** is given to close contacts of meningococcal meningitis for 2 days to prevent meningitis.
3) **Penicillin or erythromycin**: is given to individuals with abnormal heart valves prior to dental procedures to prevent endocarditis.
4) **Preoperative in some surgical operations.**



MCQs

1) **Selective toxicity of an antibiotic:**
   a) Depends on presence of a receptor for the drug in hosts not in organisms
   b) Is the ability of the drug to inhibit growth of a wide range of bacteria
   c) Depends on inhibition of a biochemical event essential for the host
   d) Is the ability of the drug to harm the organism without harming the host
   e) Is one of the complications of antibiotic therapy

2) **Which of the following antimicrobial agents is most toxic to humans?**
   a) Bacitracin
   b) Cephalosporin
   c) Amphotericin B
   d) Penicillin
   e) Vancomycin

3) **One of the following antimicrobial drugs is not among the group acting through inhibition of the bacterial cell wall:**
   a) Penicillin
   b) Vancomycin
   c) Cephalosporins
   d) Bacitracin
   e) Novobiocin

4) **Which of the following antibiotics inhibits bacterial protein synthesis by acting on the 30S ribosomal subunit?**
   a) Vancomycin
   b) Macrolides
   c) Polymyxin
   d) Aminoglycosides
   e) Chloramphenicol

5) **The following are mechanisms of acquired resistance to antimicrobial agents EXCEPT:**
   a) Decreasing the influx of the antibiotic
   b) Modification of the receptor (target) site
   c) Target elimination by developing new metabolic pathways
   d) Target overproduction
   e) Absence of cell wall

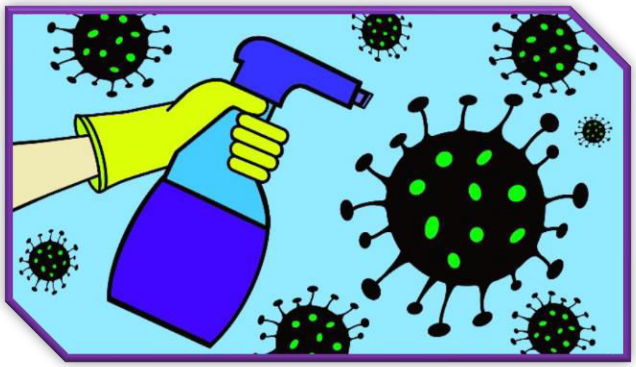
6) **The MIC is the:**
   a) Highest concentration of a drug required to inhibit bacterial growth
   b) Standard dose of a drug required to inhibit bacterial growth
   c) Lowest concentration of a drug required to inhibit bacterial growth
   d) Lowest dilution of the drug required to inhibit bacterial growth
   e) Maximum dose of a drug required to inhibit bacterial growth

7) **combined antibiotic therapy is indicated in the following conditions EXCEPT:**
   a) Mixed infections
   b) T.B.
   c) Viral meningitis
   d) Endocarditis
   e) Febrile neutropenia

8) **Regarding the effect of combined therapy with antimicrobial drugs, the expression, "1 + 1 = >2" means:**
   a) Antagonistic effect
   b) Synergistic effect
   c) Indifference
   d) Addition
   e) Ineffectiveness

Chapter 8

DISINFECTION AND STERILIZATION



Sterilization:

- Validated process used to render a product free of all forms of viable microorganisms including all bacterial spores.

- **Methods**:
  1. Steam under pressure.
  2. hydrogen peroxide gas plasma.
  3. ethylene oxide gas
  4. dry heat.

These are the main validated sterilization processes for use in the healthcare facilities.

- **Sterilization is essential for** culture media, and critical items intended to enter the vascular system and sterile tissues such as vascular catheters and surgical instruments.

Disinfection:

- It is a process that eliminates most pathogenic microorganisms except spores.
- **unlike sterilization**, disinfection is not sporicidal.

- **Disinfection is required for** devices or equipment that do not penetrate tissues but used in contact with the skin (stethoscope diaphragm swabbed with **70% alcohol**) or mucous membranes (immersion of endoscope in 2% ortho-phthalaldehyde (**OPA**) disinfectant for 12 minutes).

Disinfectant:

- Usually a chemical agent (but sometimes a physical agent) that achieves disinfection.
- ***It refers to substances applied to inanimate objects.***

Disinfectants may be categorized into 3 levels:

1. **High level disinfectant:**
   Germicide that kills all microbial pathogens except large numbers spores.
   Examples:
   - OPA for endoscopes.
   - hydrogen peroxide for contact lens.
   - chlorine for blood spills.

2. **Intermediate level disinfectant:**
   Germicide that kills all microbial pathogens except bacterial spores.
   Examples:
   - isopropyl alcohol.
   - iodophors.

3. **Low level disinfectant:**
   Germicide that kills most vegetative bacteria (except tubercle bacilli) and lipid-enveloped and medium-sized viruses such as human immunodeficiency virus and hepatitis B virus.
   Examples:
   - quaternary ammonium compounds for disinfection of floors and food preparation areas.

Antiseptic:

- A chemical disinfectant which can be safely applied to skin and mucous membranes but not suitable for systemic administration.
- The term is used especially for preparations applied topically to living tissue.
- **Examples:**
  - **70% isopropyl alcohol** to prepare skin for injection.
  - preoperative skin preparation with **alcohol-based iodine** compound in surgical operations.

Germicide:

- **Agent that destroys microorganisms.**
- **may be** → virucide, bactericide, fungicide, sporicide and tuberculocide.
- The term germicide includes both antiseptics and disinfectants.
- Antiseptics are germicides applied to living tissue and skin; disinfectants applied only to inanimate objects.

Sterilant:

Chemical germicide that achieves sterilization

Cleaning (or precleaning):

- **Removal of foreign material** (organic or inorganic contaminants) from medical devices as part of decontamination process.
- It is usually done with water and soap, detergents or enzymatic products.
- Cleaning must always precede disinfection and sterilization.

Decontamination:

- **Reduction of pathogenic microorganisms** to a level at which items are safe to handle.
- Decontamination includes sterilization and all disinfection levels.

Main Methods of Disinfection

1. **Chemical disinfectants.**

2. **Boiling water:**
   - Boiling (**100°C**) for 20 minutes achieves high disinfection.
   - It can be useful in emergencies if sterilizer is not available.

3. **Pasteurization:**
   - Pasteurization of milk by heating at **63°C for 30 min**. or at **72°C for 20 sec** → followed by rapid cooling.

- **destroys important pathogens:**
    1) Mycobacterium tuberculosis
    2) Brucella
    3) Salmonella
    4) Coxiella burnetti.

4. **Thermal disinfection by hot water:**
    - can be performed in special washing machines or linen in hospital laundry, dishes and devices which cannot withstand higher temperature.

5. **Ultraviolet radiation (UV):**
    - UV can be artificially produced by mercury lamps.
    - UV rays have weak penetration power and is used only for air and surface disinfection, → **laboratory safety cabinets**.

Methods Of Sterilization

A.Steam sterilization:

- **It is the most safe and commonly used sterilization method**.
- It is accomplished in **an autoclave** and uses moist heat in the form of saturated (**dry**) steam under pressure for a specified exposure time and at a specified temperature, as the sterilizing agent.
- **There are four parameters of steam sterilization**:
    1. Steam
    2. Pressure
    3. Temperature
    4. time.

- **The ideal steam for sterilization is saturated steam.**
- It is essential to make steam saturated which means free of air because air acts as an insulator and hinders penetration.

- **Pressure serves as** a means to obtain the high temperatures necessary to quickly kill microorganisms.

- **The two common steam-sterilizing temperatures** are **121°C** (maintained for a minimal exposure time 30 minutes) and **132°C** (maintained for 4 minutes).

- **As regard mode of action**, moist heat destroys microorganisms by coagulation and denaturation of enzymes and structural proteins.

- Steam sterilization is nontoxic, inexpensive and rapidly heats and penetrates fabrics.

- It is the most widely used and the most reliable

Monitoring of steam sterilizers (autoclaves):

1. **Mechanical indicators:**
   Using a printout or graph that monitors the time, temperature and pressure of the sterilization cycle.

2. **Chemical indicators or integrators:**
   Chemically impregnated paper strips that must be used with each sterilization cycle to monitor the temperature or time and temperature.
   Visible color changes occur at specified temperature and time.

3. **Biological indicators:**
   Paper strips impregnated with the spores of **Geobacillus stearothermophilus**.
   The biological indicators are placed at the coldest point of the chamber.
   After finishing the cycle of sterilization, spore strips are incubated in a fluid medium at 37°C for 48h.
   Absence of bacterial growth indicates an efficient sterilization cycle.

B.Low temperature sterilization methods:

1. Hydrogen peroxide gas plasma

- Gas plasmas have been referred to as **the fourth state of matter** (liquids, solids, gases, and gas plasmas).

- **Gas plasmas are generated in** an enclosed chamber under deep vacuum using radio frequency or microwave energy to excite the gas molecules and produce charged particles, many of which are in the form of free radicals.
- **The free radicals** interact with essential cell components (enzymes, nucleic acids) and thereby disrupt the metabolism of microorganisms, in addition to the direct inactivation by hydrogen peroxide.

- **Total time of sterilization cycle** is about **50 minutes**.

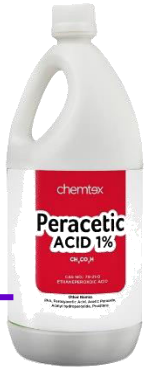
- Medical materials and devices that cannot tolerate high temperatures and humidity, such as some plastics, electrical devices, and corrosion-susceptible metal alloys, can be sterilized by this method.

- **G. stearothermophilus** (formerly Bacillus stearothermophilus) spores are used as a biological indicator to monitor efficiency of the sterilization process.

2. Ethylene oxide gas sterilization

- Exposure time is long and varies from **3 to 6 hours**.
- The method is expensive with probable toxicity.
- It can be used for instruments that cannot be subjected to steam.
- **Bacillus atrophaeus** (formerly B subtilis) spores are used as a biological indicator.

3. Peracetic acid sterilization



- **It is used to sterilize** medical, surgical, and dental instruments (endoscopes, arthroscopes).
- **Peracetic acid** denatures proteins, disrupts cell wall, and oxidizes proteins and enzymes of microbes.

C.Dry heat sterilization:

INCLUDES THE FOLLOWING FORMS:

1. Incineration:

- **is particularly applicable for** dead animal bodies, infectious hospital waste such as used surgical dressings, needles.

2. Red heat:

- Inoculating wires, loops and points of forceps are sterilized by holding them in the flame until they are red hot.

3. Dry Heat Sterilizers or hot air ovens

- The method employs dry hot air as the sterilizing agent.
- **The most common time-temperature relationships** are **170°C for 60 minutes**, **160°C for 120 minutes**, and **150°C for 150 minutes**.

- **Bacillus atrophaeus** spores should be used as a biological indicator.
- **Mode of action** → killing is due to oxidation of the microbial cell constituents
- **This method is used for** materials that might be damaged by moist heat (powders, petroleum products, sharp instruments).

- **The advantages for dry heat**:
  1. nontoxic
  2. relatively inexpensive
  3. noncorrosive for metal and sharp instruments.

- **The disadvantages**:
  1. the slow rate of heat penetration
  2. time-consuming and the high temperatures are not suitable for most materials.

D.Other sterilization methods

1. Ionizing radiation:

- Sterilization by ionizing radiation can be obtained by **cobalt 60** gamma rays or electron accelerators **(β-rays**).
- **Ionizing radiation has a high penetrating power** → used for sterilization of prepacked heat-sensitive items such as bone grafts, surgical sutures, disposable plastic syringes, gloves, catheters and plastic Petri dishes.
- **Bacillus pumilus** spores are used as a biological indicator to monitor efficiency of radiation sterilization.

2. Filtration:

- **used to** remove bacteria from thermolabile pharmaceutical fluids (antibiotic solutions, hormones, vitamins) that cannot be purified by any other means.
- Fluids can be rendered free of bacteria by passage through bacterial membrane filters with pore size as small as **0.22 µm**.
- Filters can also be used to remove microorganisms from air supplied to critical areas such as operating rooms, drug factories and laboratory biosafety cabinets. Such filters are known as high efficiency particulate air (**HEPA**) filters which can provide sterile air at the filter face.
- **The endopigment producing Serratia marcescens** may be used to test the efficiency of bacterial membrane filters.
- **spores of the fungus Aspergillus** may be used to test the efficiency of HEPA filters.

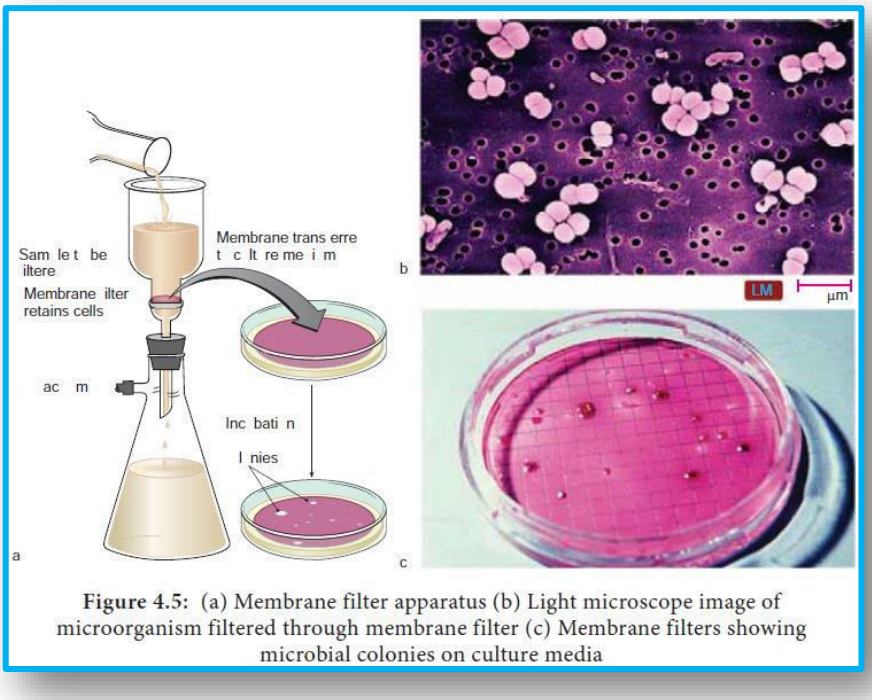


Figure 4.5: (a) Membrane filter apparatus (b) Light microscope image of microorganism filtered through membrane filter (c) Membrane filters showing microbial colonies on culture media

3. Ozone:

- Ozone (0_3) consists of 0_2 with a loosely bonded third oxygen atom that makes ozone a powerful oxidant that destroys microorganisms.
- Ozone has been used for years as a drinking water disinfectant.

4. Formaldehyde Steam:

- **Low-temperature sterilization method** that involves use of **formalin**, which is vaporized into formaldehyde gas.
- The method may be used in healthcare facilities to sterilize heat-sensitive medical equipment such as the mechanical ventilator and incubators for neonates.
- **Unfortunately**→ formaldehyde is a mutagen and a potential human carcinogen, therefore must be regulated and fully contained to guarantee the permissible exposure limit of healthcare workers for formaldehyde.

5. Infrared radiation

MCQs

1) One of the following statements is CORRECT:

a) Sterilization is complete removal or inactivation of all forms of microbial life.
b) Disinfection is elimination of all pathogenic organisms including spores.
c) Low level disinfection is effective against Mycobacterium tuberculosis.
d) Antiseptics are chemical disinfectants applied to surfaces and floors.
e) High level disinfection is enough for surgical instruments and needles.

2) Pasteurization:

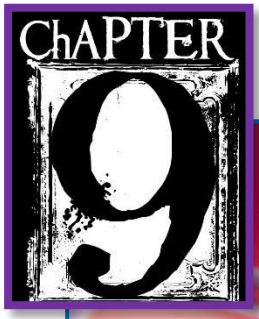
a) Is generally performed at 87°C for 30 minutes.
b) Can destroy important pathogenic organisms.
c) Is a method of sterilization.
d) Is done by hot water at temperatures higher than 100°c
e) Cannot destroy Mycobacterium tuberculosis.

3) Regarding hot air oven:

a) It is used to sterilize powders and petroleum products.
b) The sterilizing agent is moist heat.
c) It has a corroding effect.
d) It doesn't necessitate prolonged exposure.
e) It is characterized by rapid and even penetration of heat into the materials to be sterilized.

4) Regarding biological indicators for monitoring autoclaves:

a) They are placed at the hottest part of the chamber.
b) They change their color at 121°C
c) They are paper strips containing the spores of G. stearothermophilus.
d) Presence of bacterial growth indicates an efficient sterilization cycle.
e) They are checked for color change at the end of each sterilization cycle



BACTERIAL PATHOGENESIS

Infection: a process by which the organism enters into a relationship with the host.

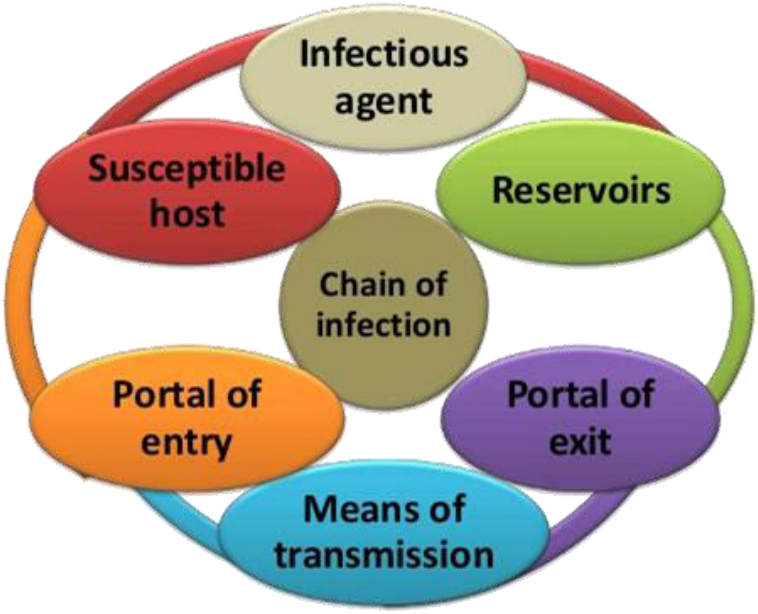
- ☹ **Although microbial infections occur frequently**, most infections end without occurrence of pathological changes and thus are not manifested as clinical disease.
- ☹ **These infections are termed**:
  1) Subclinical
  2) silent
  3) abortive infections.

The outcome of bacterial infections depends on the mutual relationship between bacteria and host.

Bacteria could be classified into:

1. **Saprophytic bacteria:**
   - are those which live freely in nature, on decaying organic matter, in soil or water.
   - They do not require a living host.

2. **Parasitic bacteria:**
   - are those which live on or in a living host.
   - **They are classified according to their relation to the host into**:
     a) **Pathogenic**: Bacteria capable of causing disease.
     b) **Non-pathogenic** (**commensals**): Bacteria that do not cause disease, and are part of the normal flora.
     c) **Opportunistic pathogens**: These are potentially pathogenic bacteria that do not cause disease under normal conditions but can cause disease in immunocompromised patients, or when they find their way to another site other than their normal habitat.
     Many of these opportunistic pathogens are originally commensals.



Stages of the Infectious Process

1) **Source of infection** which may be man (**case or carrier**), animal or soil.
2) **Mode of transmission** → droplet inhalation, ingestion, injection, insects, contact and transplacental.
3) **Portal of entry** → respiratory tract, gastrointestinal tract, skin.
   The organism then starts to multiply within the host causing tissue damage (**disease**).
4) **Portal of exit** → urine, stools, blood, respiratory or genital discharge, from which the organism is transmitted to a new host.

Koch's postulates.

- These are criteria that were proposed by Koch in order to determine if the organism
- isolated from the patient actually caused the disease.
- these criteria must be satisfied to confirm the causal role of an organism.

- **These criteria are as follows:**

1. The organism must be Isolated from every patient with the disease.
2. The organism must be Isolated free from all other organisms and grown in pure culture in vitro.
3. The pure organism must cause the disease in a healthy, susceptible animal.
4. The organism must be recovered from the inoculated animal.

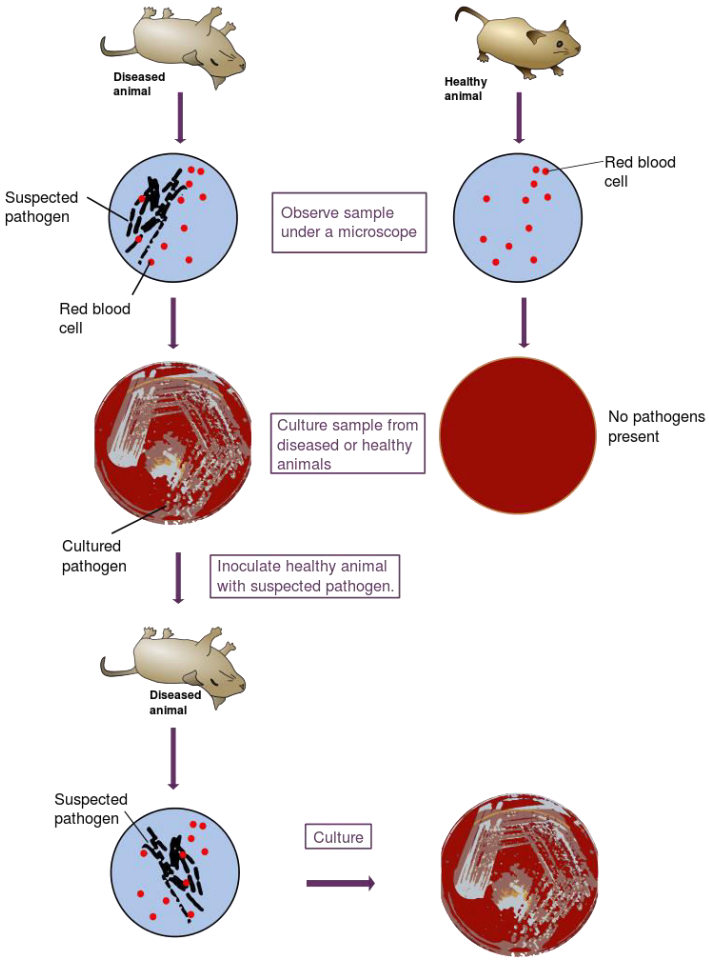
Koch's Postulates:

① The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

② The microorganism must be isolated from a diseased organism and grown in pure culture.

③ The cultured microorganism should cause disease when introduced into a healthy organism.

④ The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.



The outcome of infection depends on the interaction between microbial factors (virulence) and host resistance factors (Immunity).

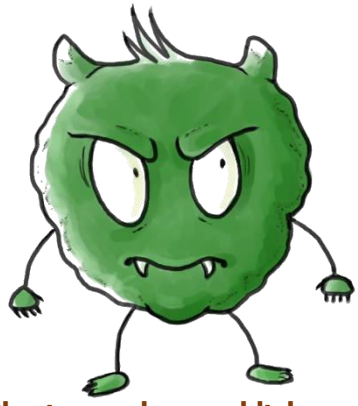
MICROBIAL VIRULENCE

Pathogenicity: a qualitative description of a species of bacteria denoting ability to produce disease.

Virulence: a quantitative character (degree of pathogenicity) of a strain belonging to a pathogenic species.

Virulence Is genetically determined by genes carried on plasmids, phages, pathogenicity Islands and chromosomes.

Virulence Factors of Bacteria



A virulence factor is either a structure (capsule) or a product (toxins) that enables the organism to cause disease.

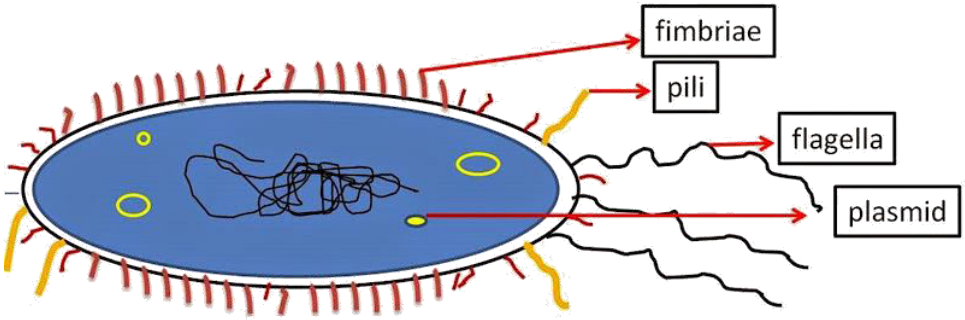
A - Adherence factors

They enable bacteria to attach to the host surfaces, thus contributing to the establishment of the infection.

example:

1. The fimbriae of **Neisseria gonorrhoeae** and **E. coli** help the attachment of these organisms to the urinary tract epithelium.
2. The glycocalyx of **Staphylococcus epidermidis** and certain **viridans streptococci** allows the organisms to adhere strongly to the heart valves.

Mutants that lack these factors are often avirulent.



B - Invasion factors

Invasion of tissue followed by inflammation is one of the main mechanisms by which bacteria can cause disease.

This invasion is helped by:

A. Enzymes

1) **Collagenase and hyaluronidase** which degrade collagen and hyaluronic acid and allow the bacteria to spread through subcutaneous tissues.
2) **immunoglobulin A protease** which degrades IgA.
3) **Leukocidin** which can destroy both polymorphonuclear leucocytes and macrophages.
4) **Deoxyribonuclease** that breaks down DNA.
5) **Lecithinase** that breaks down lecithin of cell membrane

B. ANTIPHAGOCYTIC FACTORS

1) **Capsule**: The capsule prevents the phagocytes from attachment to the bacteria → **Strept. pneumoniae**.

2) **Cell wall proteins of Gram-positive cocci**, such as the M protein of **Strept. pyogenes** and protein A of **Staph. aureus**.

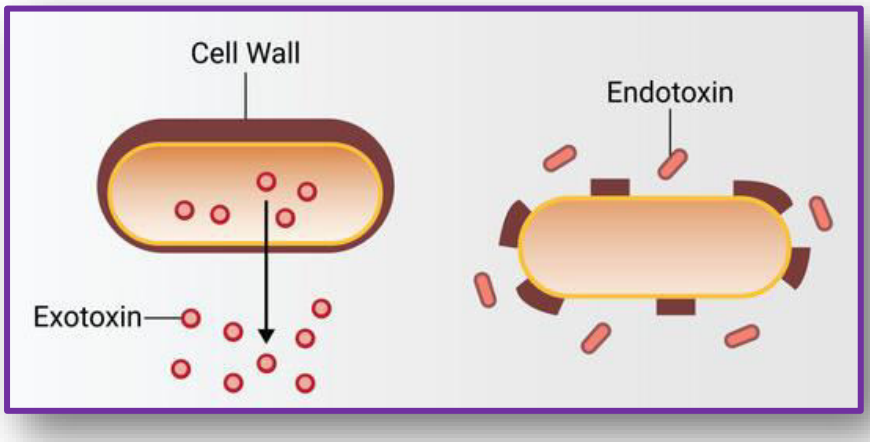
3) **Coagulase**: It accelerates the formation of a fibrin clot from fibrinogen. This clot can protect bacteria from phagocytosis → **Staph. aureus**.

C. TOXIN PRODUCTION

Toxin production is another mechanism by which bacteria can produce disease.

BACTERIAL TOXINS ARE EITHER EXOTOXINS OR ENDOTOXINS.

	Exotoxins	Endotoxins
Source	Secreted by both gram positive and gram negative	Part of cell wall of gram-negative bacteria. Liberated upon cell disintegration.
Coding genes	Encoded by chromosomes, plasmids, bacteriophages or PAIs.	Encoded by genes on the chromosome.
Examples	**C. diphtheria** (**phage**) **Cl. Tetani** (**plasmid**) **B. pertussis** (**chromosome**) **H. pylori** (**PAI**)	**E. coli** **Meningococci**
Nature	protein	Lipopolysaccharide (**Lipid A**)
Antigenicity	Highly antigenic	Poorly antigenic
Heat stability	Unstable to temperature above **60 C**	Stable to temperature above **60 C** for several hours.
Detoxification	Can be converted into **Toxoids**.	Cannot
Specificity	Every toxin has specific action	General effect (**septic shock**)
Toxicity	High	Low



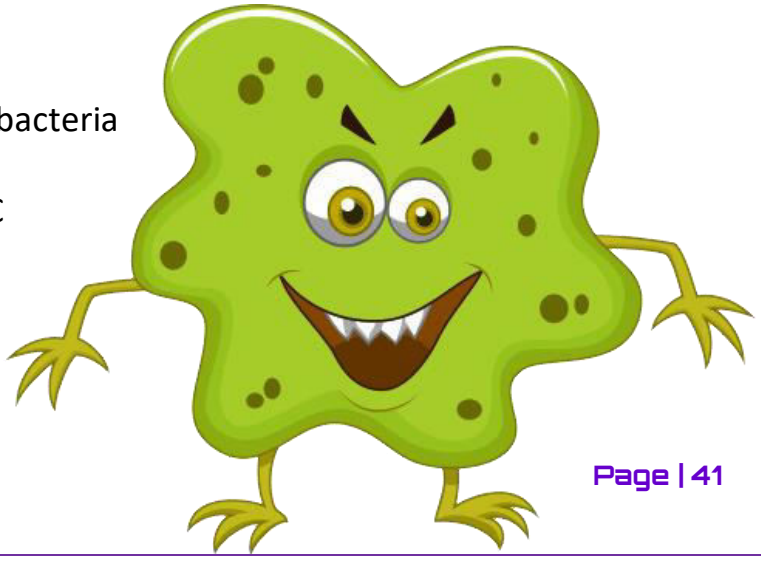
Treatment of exotoxin with formalin removes its toxicity and retains its antigenicity converting it into toxoid, that can be used for immunization.

MCQs

1. **Opportunistic pathogens:**
   a) Are never the cause of a clinical infection
   b) Are usually highly pathogenic
   c) Are rarely part of the normal flora
   d) Cause disease mainly in immunocompromised individuals
   e) Are resistant to killing by steam sterilization

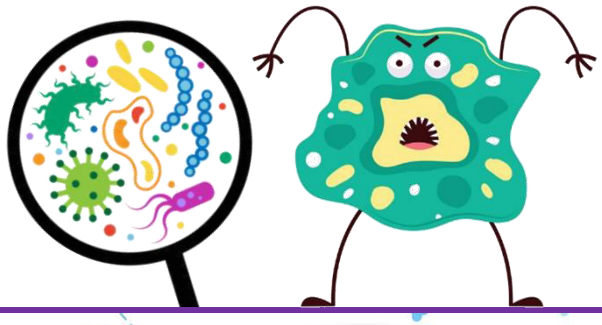
2. **Exotoxins have the following characters, EXCEPT:**
   a) They may be encoded by genes on the chromosome.
   b) They can be converted to toxoids.
   c) They have specific action.
   d) They are polypeptides.
   e) They are heat stable.

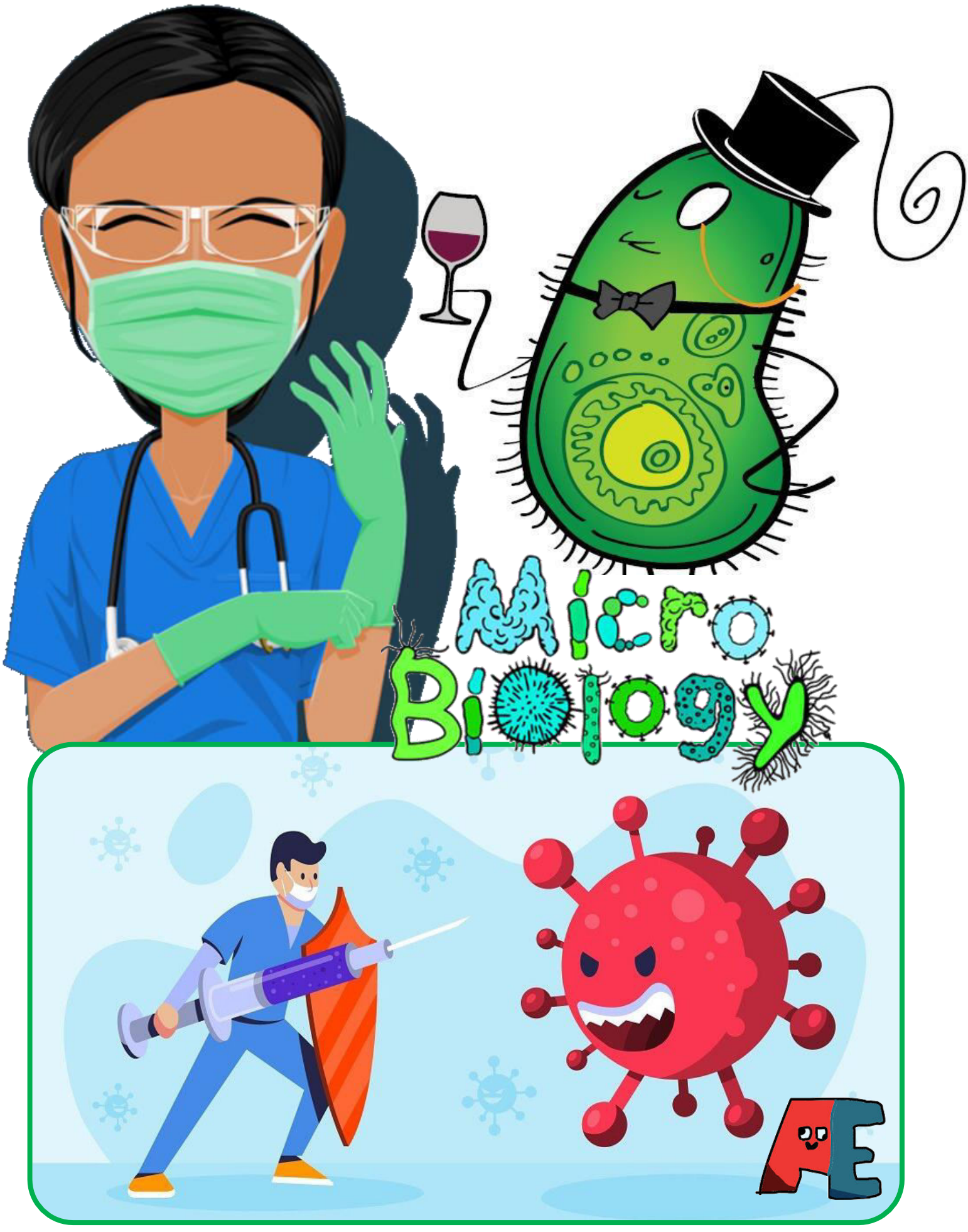
3. **Endotoxins:**
   a) Are secreted mainly by Gram-positive bacteria
   b) Are highly antigenic
   c) Are stable at temperatures above 60°C
   d) Can be converted into toxoid
   e) Have specific action



General MICROBIOLOGY

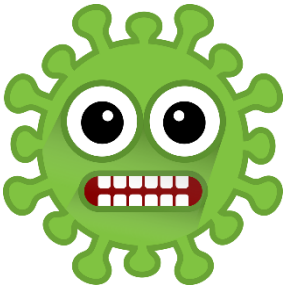
PART 3




Micro
Biology

Chapter 10

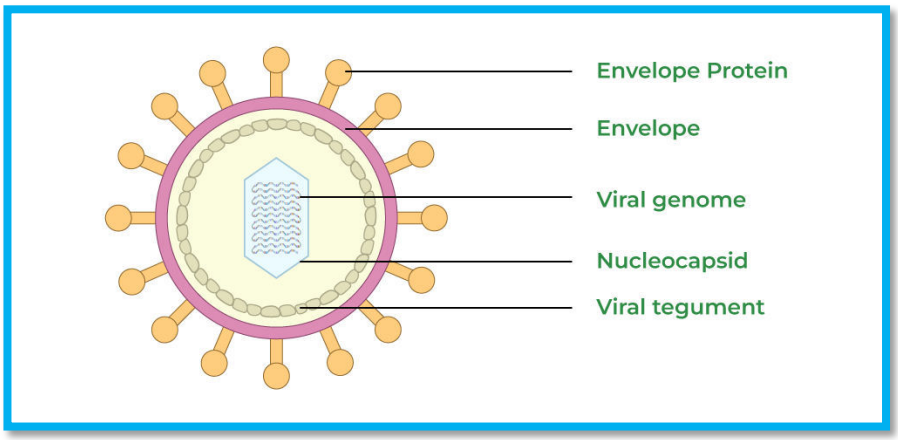
GENERAL VIROLOGY



- Viruses are one of the smallest infectious agents.
- They are obligatory intracellular parasites because they have no metabolic activity.
- **Viruses can infect all organisms in nature:**
  1. **Bacteriophages**: are bacterial viruses.
  2. **Plant viruses**: include complete viruses and viroids.
  3. **Animal viruses**: infect insects or vertebrates including man.

Viruses differ from bacteria in the following:

1. **Viruses are very small** in size, ranging from **20-300 nm**.
   Therefore: They can only be seen under the electron microscope (except poxviruses).
   They can pass through bacterial filters.
   They need ultracentrifugation for sedimentation.
2. **Viruses contain only one type of nucleic acid** (DNA or RNA), never both.
3. **They are obligatory intracellular parasites** (can only replicate inside living cells) and do not divide by binary fission.
4. **They cannot be cultivated in the laboratory** on artificial culture media → they can be grown on tissue culture.
5. **They are not susceptible to antibacterial antibiotics**.



Structure and Composition of Viruses

- The typical complete virus particle, called virion, consists of a genome of either DNA or RNA, surrounded by a capsid (protein coat).
- The nucleic acid and the protein coat are called nucleocapsid.
- Some viruses, called enveloped viruses, have an outer Lipid containing envelope whereas others are non-enveloped (naked).

Viral nucleic acid (genome)

- It is the genetic material of a virus, which may be either RNA or DNA.
- Most DNA viruses are double-stranded (**ds**) while most RNA viruses are single stranded (**ss**).
- The viral ssRNA may be positive sense strand **(+sense)** or negative sense strand **(-sense)**.
- **It is responsible for virulence** → it is the infectious part of the virus.

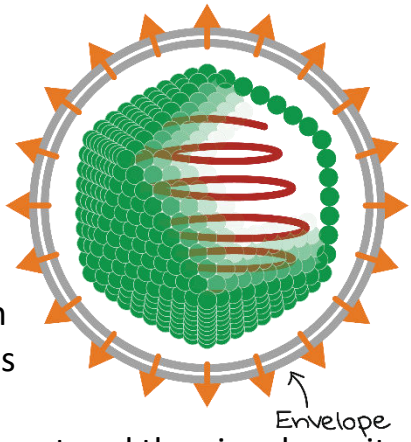
Viral capsid

Viral capsid is composed of many small protein subunits called capsomers.

Functions:

1. **It protects the nucleic acid** (genome) against harmful environmental factors.
2. **It mediates attachment** to host cell (in non-enveloped viruses}.
3. **It is responsible for the viral symmetry** (or morphology} **WHICH MAY BE**:
   a) **Icosahedral (many sided) symmetry**: Icosahedral or isomeric or cubic viruses resemble a crystal with 20 triangular facets and 12 comers.
   This includes all DNA viruses, except ***poxviruses*** (brick shaped), and some RNA viruses.
   b) **Helical (coiled tubes) symmetry**: The viral nucleic acid is closely associated with the protein capsid forming a coil-shaped helical nucleocapsid.
   This includes many of RNA viruses → ***rabies virus***.
   c) **Complex symmetry**: Examples include the brick-shaped poxviruses or ***bacteriophages***.

Viral envelope



- It is a lipoprotein membrane composed of lipids, derived from host cell membrane during release by budding, and protein that is virus-specific.
- **the envelope may have glycoprotein spikes** which are the organ of attachment of the enveloped virus to host cell receptors.
- Therefore, dissolving the envelope inhibits attachment and the virus loses its infectivity.
- **Enveloped viruses are less stable** → more easily inactivated than naked viruses.
- They are more sensitive to heat, drying, detergents and lipid solvents.
- **enveloped viruses**, being unable to survive in the environment, are transmitted essentially by direct contact via blood and body fluids.

The surface proteins of the virus, whether they are the capsid proteins (in naked viruses) or the glycoproteins (in enveloped viruses) are responsible for attachment to host cell receptors
the principal antigens against which the host elicits its immune response to viruses.

Classification of Viruses

XXX

a) **Classification by symptomatology** It is the old classification based on diseases that viruses produce → **tropism** → neurotropic viruses, enteroviruses.

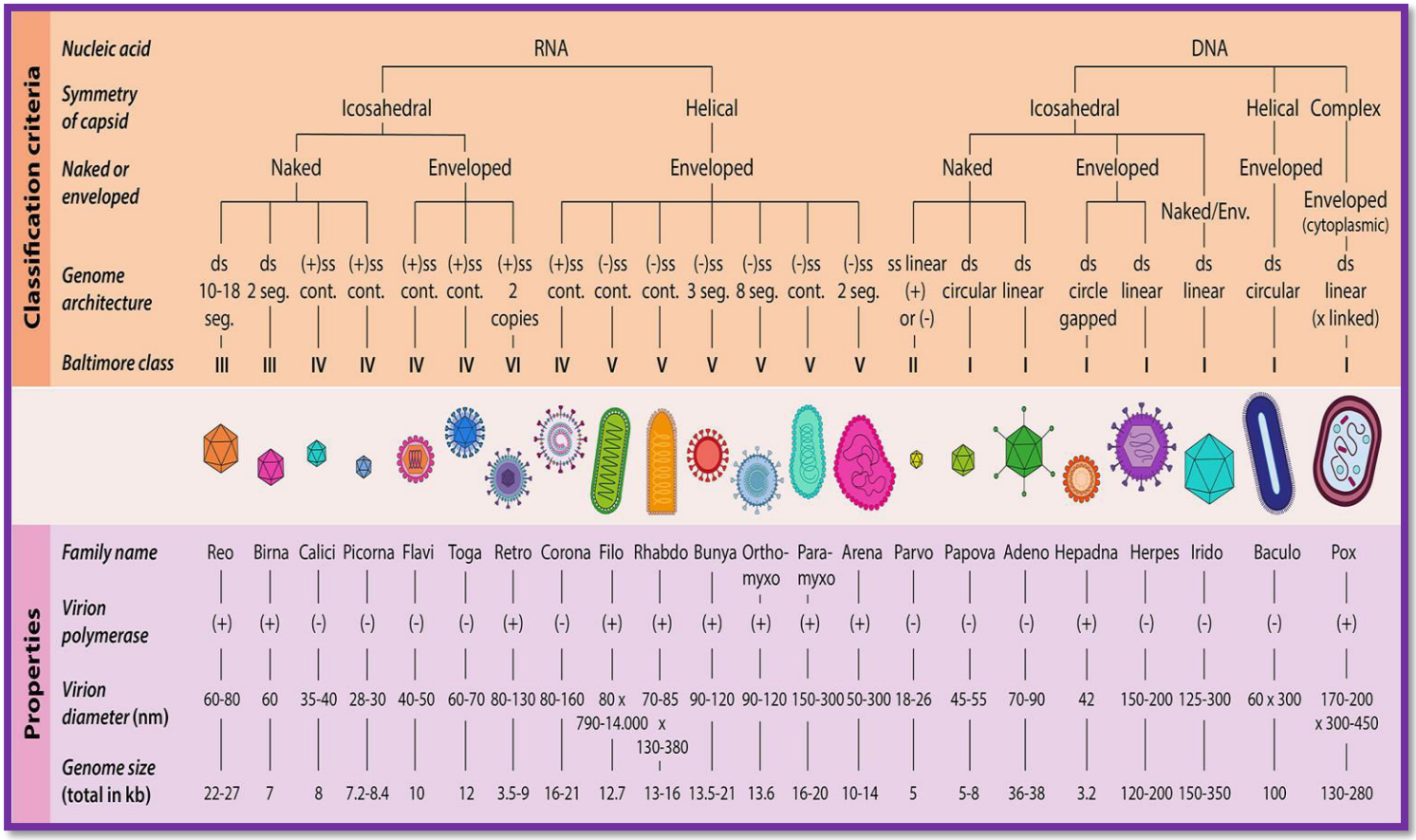
b) The hierarchical virus classification

THE SCHEME CLASSIFYING VIRUSES INTO ORDERS, FAMILIES AND SUBFAMILIES IS BASED ON:

1. **Nature of the nucleic acid**: RNA or DNA genome.
2. **Virus replication strategy**
3. **Symmetry of the capsid**
4. **Presence or absence of an envelope** Further classification is based on additional properties → antigenicity, host range and nucleic acid sequence.

c) The Baltimore classification:

- It is based on virus genome replication strategy.
- The central idea is that all viruses must generate positive strand mRNAs from their genomes, in order to produce proteins and replicate themselves.
- The precise mechanisms whereby this is achieved differ for each virus family.



Virus Replication

- Viruses are unable to replicate on their own because they lack the genes and enzymes necessary for energy production.
- replication depends on living host cells and is directed by the viral genome to produce the virus components.

Viral replication occurs in the following steps.

1. Attachment or adsorption:

- Adsorption of the virus occurs to specific receptor sites on the surface of the susceptible host cell.
- These interactions determine viral host range (human viruses and plant viruses) and tissue specificity or tropism (hepatotropic viruses and neurotropic viruses).

2. Penetration:

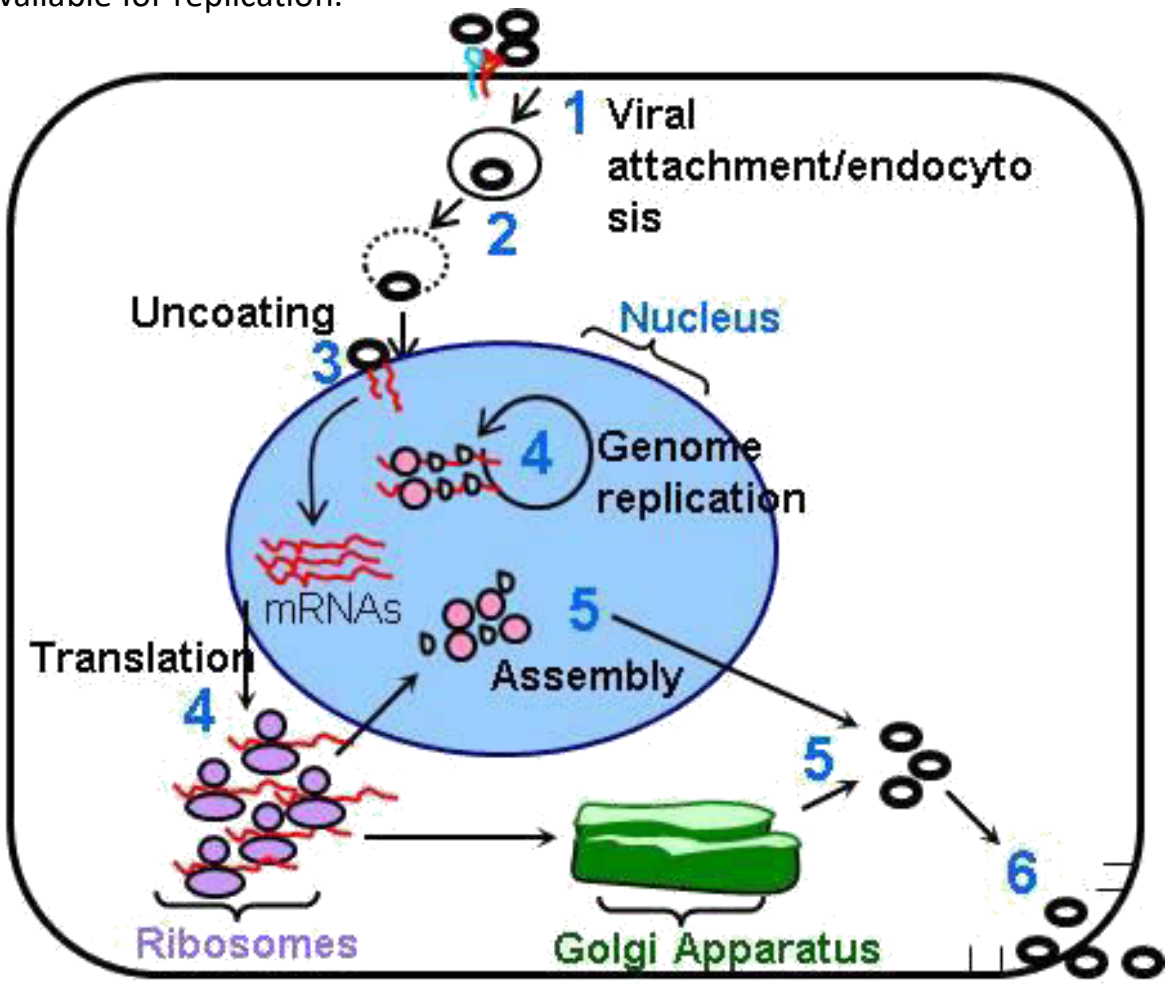
The viruses may enter the host cells by either:

a- **endocytosis** in case of non-enveloped viruses.

b- **fusion** of viral envelope with host cell membrane in case of enveloped viruses.

3. Uncoating:

The nucleic acid is released from the capsid by the action of cellular enzymes and becomes available for replication.



4. Synthesis of viral components

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A) Synthesis of viral proteins:

Transcription:

- Viruses must first synthesize virus-specific messenger RNA (mRNA) to synthesize virus specific proteins.

- Transcription of mRNA varies according to the type of viral nucleic acid whether DNA or RNA, ds or ss, positive or negative sense strand, AS FOLLOWS:

a) DNA viruses: mRNA can be transcribed from the negative sense strand using the host's DNA-dependent RNA polymerase.

b) RNA viruses: There is no host cell RNA polymerase that can use the viral RNA as a template for synthesis of mRNA.
RNA viruses fall into 4 groups according to the strategy for synthesizing mRNA→

In dsRNA viruses, the negative sense strand is transcribed by viral RNAdependent RNA polymerase into mRNA.

In ssRNA viruses THERE ARE 3 DISTINCT ROUTES TO THE FORMATION OF MRNA:

i. The strand with positive sense acts directly as mRNA.

ii. The strand with negative sense must first be transcribed, using viral RNA-dependent RNA polymerase, into positive sense strand which can then act as mRNA.

iii. In retroviruses, the positive ssRNA is first made into a negative sense ssDNA using the viral reverse transcriptase.
Then dsDNA is formed by the host DNA-dependent DNA polymerase.

This dsDNA enters the nucleus and is either:

a) transcribed by host's DNA-dependent RNA polymerase into mRNA or
b) integrated in host cell genome causing transformation.

Translation:

Once the viral mRNA is transcribed, it is translated using host ribosomes to synthesize viral proteins.

B) Synthesis of viral nucleic acid:

Replication of the viral genome requires the synthesis of a strand with a complementary base sequence, which serves as the template for the synthesis of several copies of the original viral genome.

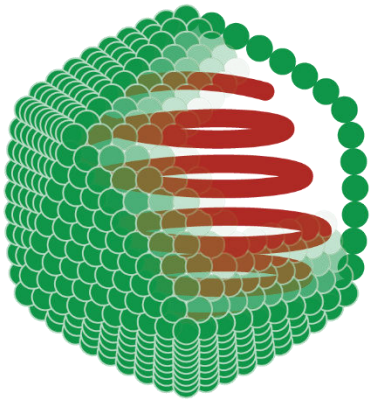
5. Assembly:

- The newly synthesized protein coats enclose the replicated nucleic acids to form mature viruses (**virions**).
- **This occurs either in the nucleus of the host** cell → ***herpes viruses***
  or in the cytoplasm → ***polioviruses***.

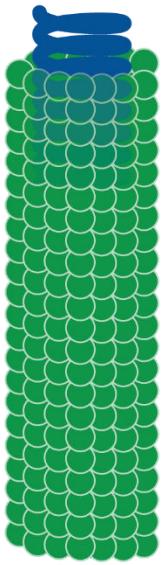
6. Release: THE NEW VIRUSES ARE RELEASED EITHER BY:

a) **lysis** of host cell in case of non-enveloped viruses → ***poliovirus***
b) **budding** through the cell membrane in case of enveloped viruses →***HIV.***

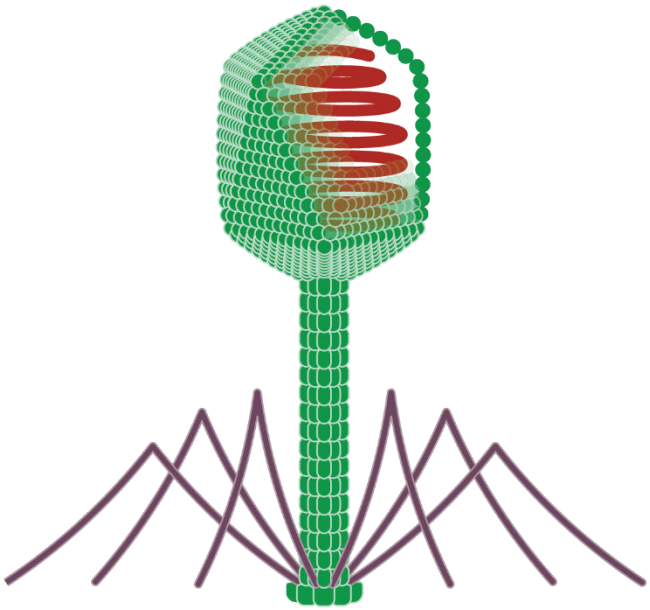
Eclipse is the time from uncoating until assembly of mature viruses.
During this phase, no infectious viruses can be detected in the host cell.
Some viruses do not initiate synthesis and remain latent within the host cell for variable periods



Icosahedral



Filamentous



Head-tail

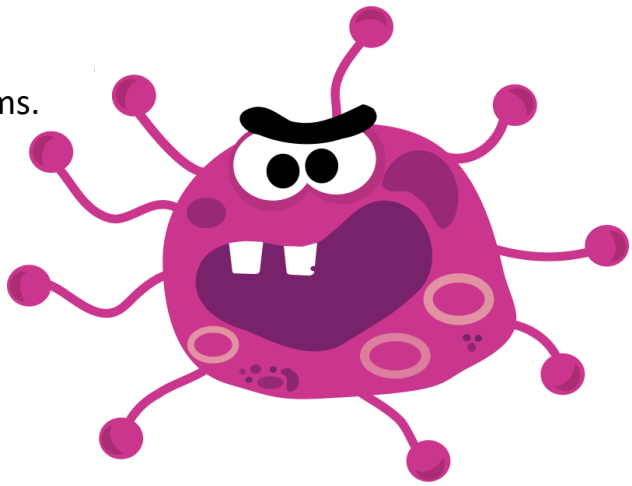
Pathogenesis of Viral Diseases

Entry of viruses

- **Viruses enter the body either by** inhalation (respiratory tract), ingestion (GIT), contact (urogenital system) or through skin (injections, blood transfusion, insect and animal bites).
- **VIRAL INFECTION MAY BE:**
  a) **Local infection**: where the virus produces disease at the portal of entry.
  b) **systemic or deep viral infections**: where the virus spreads to distant organs either via the blood (viraemia), or by other means (along nerves).

	Local infections	Systemic infections
Example	Common cold	Measles
Site of pathology	Portal of entry	Distant sites
Incubation period	Short	Long
Viremia	Absent	Present
Duration of immunity	Short	Life long
Immunoglobulins	IgA	IgM - IgG

Fate of viral infections



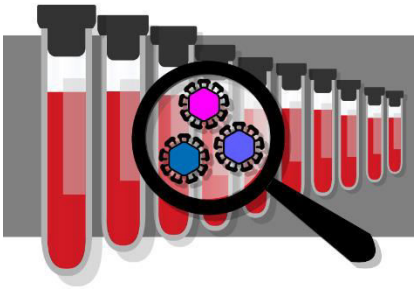
1. **inapparent or subclinical viral infections**:
   Viral infection without overt signs and symptoms.
2. **Apparent infections** (**disease**):
   Local or systemic viral infections with the appearance of clinical signs and symptoms.
3. **Persistent viral infections** (**chronic**):
   the virus is continuously detected with mild or no clinical symptoms, → ***chronic hepatitis B***.
4. **Latent viral infections**:
   The virus persists in a dormant form and may flare up intermittently to produce disease → ***herpes viruses***.
5. **Slow virus infections**:
   Virus infections with long incubation periods (months or years).

THEY ARE CAUSED BY TWO TYPES OF INFECTIOUS AGENTS:

1. **Conventional viruses** →
   a variant of measles virus which causes subacute sclerosing panencephalitis (***SSPE***).
2. **Unconventional agents** (***prions***).

Laboratory Diagnosis of Viral Infections

The laboratory diagnosis of viral infection involves 2 main diagnostic methods:



A- **Direct methods**: which depend either on the detection of viruses and/or their components in the patient's specimens, or on isolation of viruses.

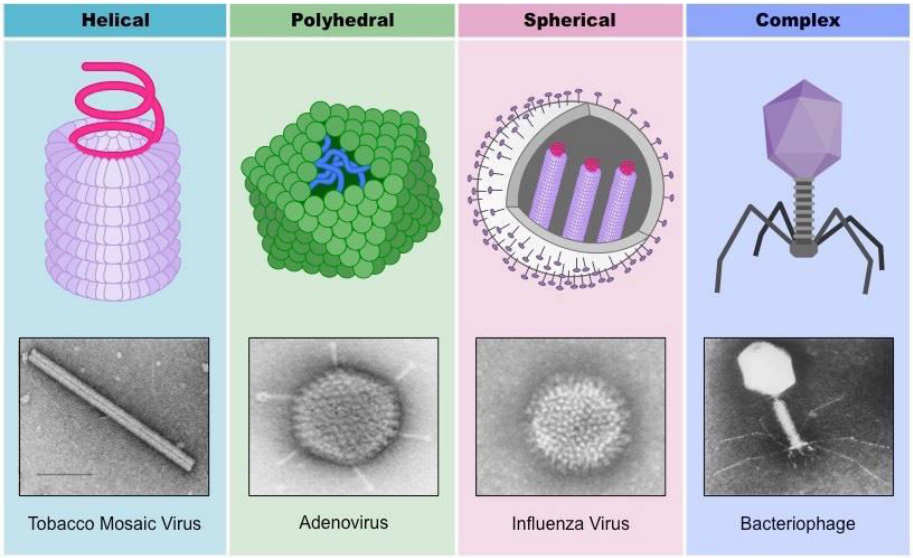
B- Indirect methods: which depend mainly on the detection of antibodies against the suspected virus in the patient's serum, or on skin tests.

Treatment of Viral Infections Viruses

- ☹ **cannot be treated with antibiotics** because they lack the structural targets on which antibiotics can act.
- ☹ **Viruses are obligate intracellular parasites**, so antiviral drugs must selectively inhibit viral replication without causing damage to host cells.
- ☹ The number of antiviral drugs is little compared to antibacterial drugs.

Mechanisms of antiviral drugs:

1- Fusion inhibitors → block virus entry.
2- Uncoating inhibitors → inhibit virus uncoating.
3- Neuraminidase inhibitor → interfere with viral release from infected cells.
4- Nucleoside analogues → inhibit DNA polymerase → inhibit DNA synthesis
5- Inhibitors of mRNA Synthesis
6- Reverse transcriptase inhibitors.
7- Protease inhibitors.
8- Inhibitors of viral protein synthesis.



MCQs

1- Viruses differ from bacteria in the following EXCEPT:

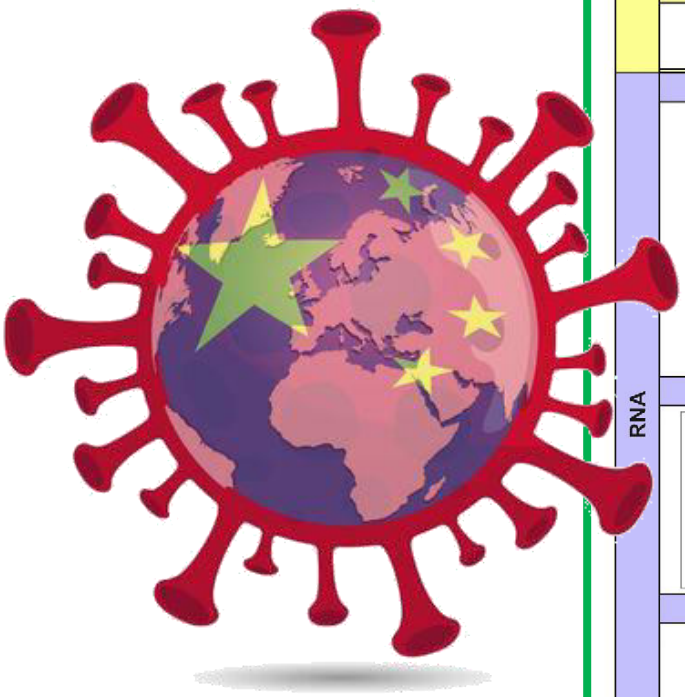
a- They are very small in size.
b- They contain two types of nucleic acid.
c- They are obligatory intracellular parasites.
d- They need ultra-centrifugation for their sedimentation.
e- They can be seen only by the electron microscope.

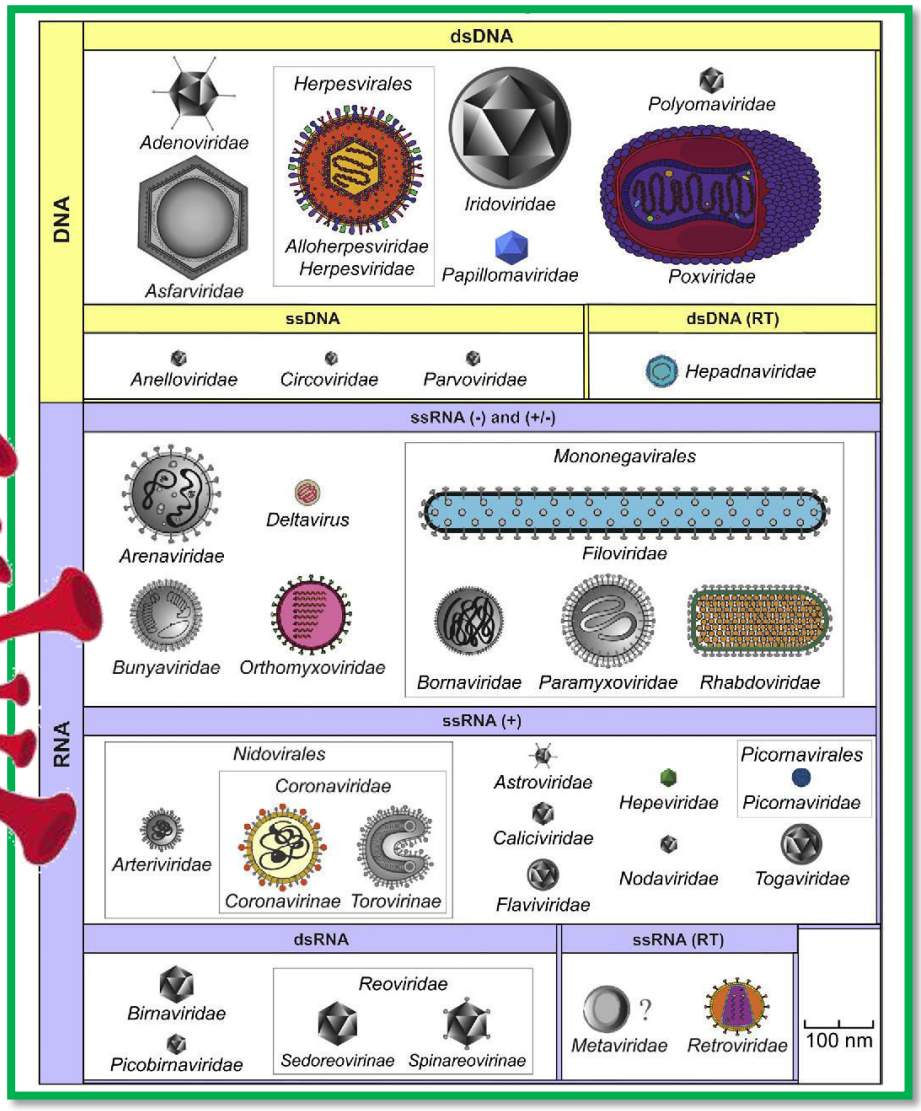
2- All of the following is true concerning viral capsid EXCEPT:

a- It is formed of capsomers.
b- It is protein in nature.
c- It is responsible for viral symmetry.
d- It is the infectious part of the virus.
e- It protects the nucleic acid.

3- Local viral infections are characterized by:

a- Long incubation period
b- Short duration of immunity
c- Insignificant role of IgA
d- Important role of IgM and IgG
e- A stage of viraemia



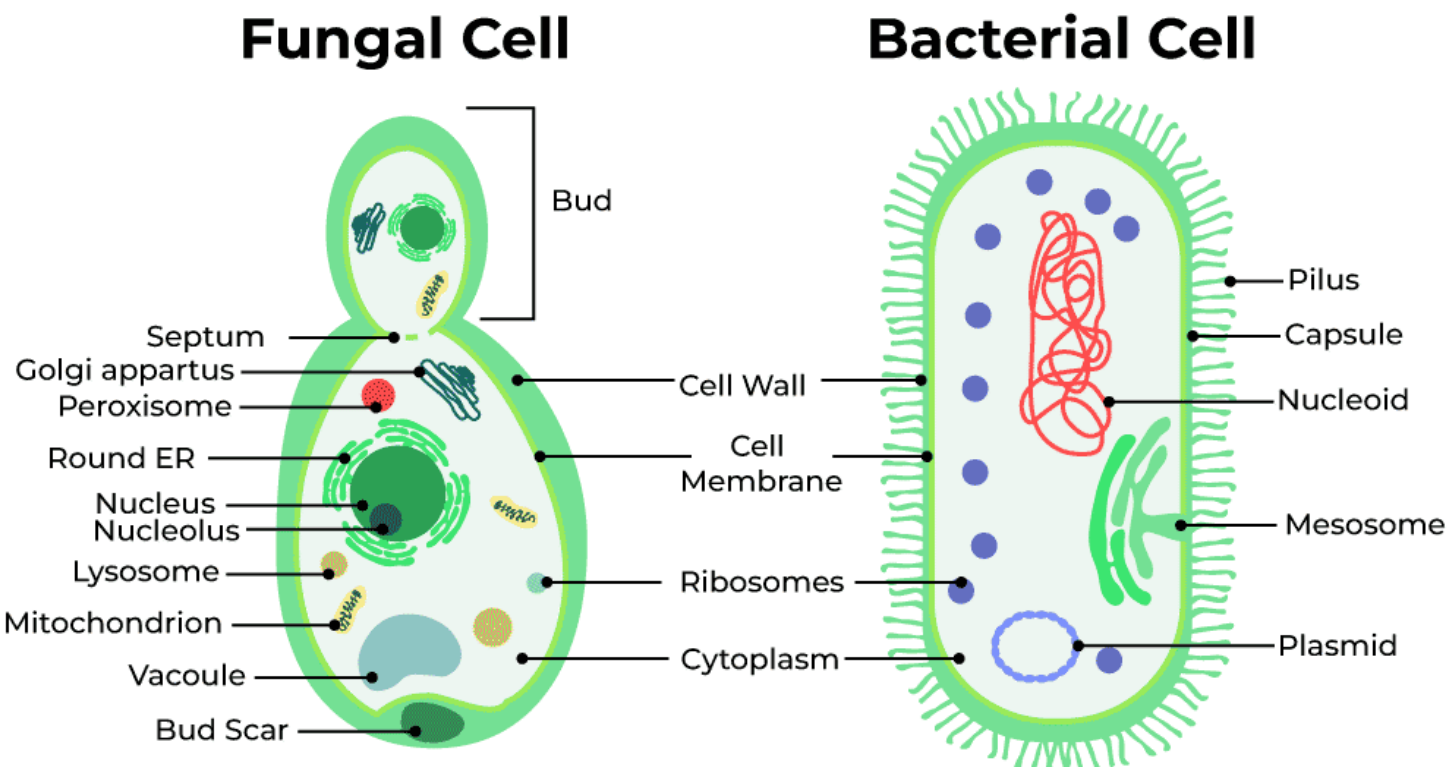


CHAPTER 11

GENERAL MYCOLOGY

Mycology is the study of fungi.

	Fungi	Bacteria
Size	Larger	Smaller
Nucleus	Eukaryote	Prokaryote
Mitochondria	Present	Absent
Ergosterol in cytoplasmic membrane	Present	Absent
Cell wall	Chitin	Peptidoglycan
Spores	For reproduction	For survival
Metabolism	Heterotrophic No obligate anaerobe	Hetero and autotrophic Many obligate anaerobes



Mycoplasma is the only bacteria that contains cholesterol in the cytoplasmic membrane

Reproduction may be by both sexual (meiotic) or asexual (mitotic) spores

Morphological Forms:

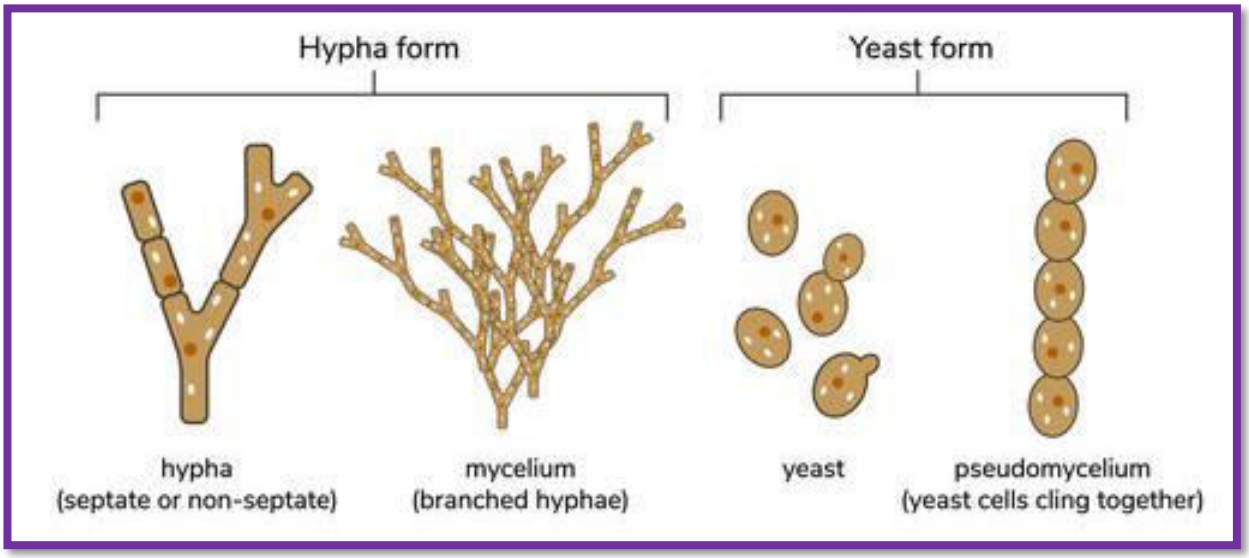
1- Molds:

They consist of long filaments (hyphae) which may be:

a) **Septate** (with cross walls)
b) **Non-septate** (without cross walls).

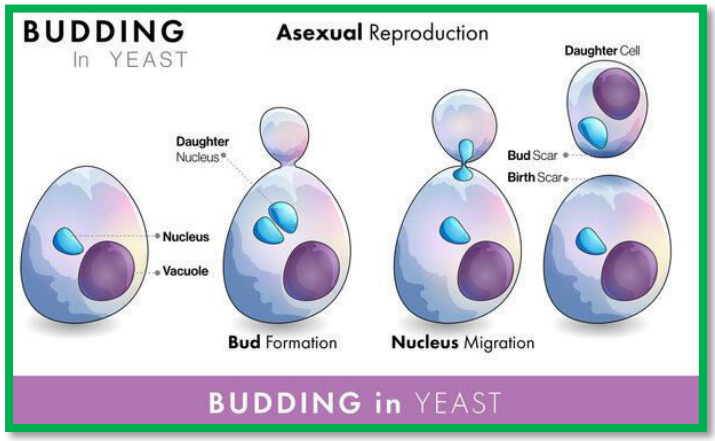
They grow by branching and tip elongation forming a mass called mycelium on culture media

Examples → ***Aspergillus***, ***Penicillum*** and the ***dermatophytes***.



2- Yeasts:

- They grow as single cells (round or oval).
- **They reproduce by budding** and may form pseudo-hyphae (hyphae with constrictions → sausage-like chain)
- **Examples** → ***Candida*** and ***Cryptococcus***.



3- Dimorphic fungi

those that can switch between the previous two forms depending on the temperature:

a) **At room temperature**: they grow as molds (hyphae).
b) **At body temperature**: they grow as yeasts.

Example → ***Histoplasma***

Clinical Classification:

A- Mycotic infections

1. Superficial mycoses: affecting the keratinized layer of the skin → ***Pityriasis versicolor***.

2. Cutaneous mycoses: affecting the deep layers of the skin → ***candida*** and ***dermatophytes***.

3. Subcutaneous mycoses: in which fungi present in the soil are implanted in the subcutaneous tissue by trauma, → ***mycetoma***.

4. Deep (systemic) mycoses: affecting internal organs.

THESE FUNGI FALL IN TWO GROUPS:

a) **"True pathogens"** infecting normal healthy individuals → ***Histoplasma*** and ***Blastomyces***.

b) **"Opportunistic pathogens"** infecting immunocompromised individuals → ***Pneumocystis***, ***Cryptococcus*** and ***Candida***.

B-Mycotoxicosis:

It is produced by consumption of food containing fungal toxins

Examples →

a) **Mushroom poisoning** causes damage to liver, kidney and bone marrow.
b) **Aflatoxin** of Aspergillus flavus may cause chronic liver damage and cancer.

C- Allergic disorders: Spores of free-living fungi as Aspergillus may be the allergen in some cases of atopy (asthma, hay fever, urticaria).

Pathogenesis

- **Infection with certain systemic fungi** (***Histoplasma***) elicits a granulomatous host defense response (composed of macrophages and helper T cells).
- **infection with other fungi** (***Aspergillus***) elicits a pyogenic response (composed of neutrophils).

Diagnosis of fungal infections

The laboratory diagnosis of fungal infection involves 2 main diagnostic methods:

A- Direct methods: which depend either on the detection of fungi and/or their antigens in the patient's specimens, or on isolation of fungi.

B- Indirect methods: which depend mainly on the detection of serum antibodies against the suspected fungus in systemic mycosis or, less frequently, on skin tests

Antifungal Drugs:

- **Because fungi are eukaryotes**, the range of non-toxic systemically active antifungal drugs is still limited.

- **The selective toxicity of antifungal drugs** is based on the presence of ergosterol in fungal cell membranes, in contrast to the cholesterol found in human cell membranes and the absence of sterols in bacterial cell membranes.

- **The most commonly used drugs are →**
  1. **amphotericin B**
  2. **nystatin**
  3. **azole drugs** (fluconazole, ketoconazole and itraconazole).

MCQs

1- Fungi have the following characters EXCEPT:

a- They replicate sexually and asexually.
b- They are eukaryotic.
c- They have ergosterol in the cell membrane.
d- They are heterotrophic.
e- They are susceptible to antibacterial agents.